BONUS BIO-C3

Biodiversity changes: causes, consequences and management implications

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BIO-C3 overview

The importance of biodiversity for ecosystems on land has long been acknowledged. In contrast, its role for marine ecosystems has gained less research attention. The overarching aim of BIO-C3 is to address biodiversity changes, their causes, consequences and possible management implications for the Baltic Sea. Scientists from 7 European countries and 13 partner institutes are involved. Project coordinator is the GEOMAR Helmholtz Centre for Ocean Research Kiel, Germany, assisted by DTU Aqua, National Institute of Aquatic Resources, Technical University of Denmark.

Why is Biodiversity important?

An estimated 130 animal and plant species go extinct every day. In 1992 the United Nations tried countering this process with the "Biodiversity Convention". It labeled biodiversity as worthy of preservation – at land as well as at sea. Biological variety should not only be preserved for ethical reasons: It also fulfils key ecosystem functions and provides ecosystem services. In the sea this includes healthy fish stocks, clear water without algal blooms but also the absorption of nutrients from agriculture.

Biodiversity and BIO-C3

To assess the role of biodiversity in marine ecosystems, BIO-C3 uses a natural laboratory: the Baltic Sea. The Baltic is perfectly suited since its species composition is very young, with current salt level persisting for only a few thousand years. It is also relatively species poor, and extinctions of residents or invasions of new species is therefore expected to have a more dramatic effect compared to species rich and presumably more stable ecosystems.

Moreover, human impacts on the Baltic ecosystem are larger than in most other sea regions, as this marginal sea is surrounded by densely populated areas. A further BIO-C3 focus is to predict and assess future anthropogenic impacts such as fishing and eutrophication, as well as changes related to global (climate) change using a suite of models.

If talking about biological variety, it is important to consider genetic diversity as well, a largely neglected issue. A central question is whether important organisms such as zooplankton and fish can cope or even adapt on contemporary time scales to changed environmental conditions anticipated under different global change scenarios.

BIO-C3 aims to increase understanding of both temporal changes in biodiversity - on all levels from genetic diversity to ecosystem composition - and of the environmental and anthropogenic pressures driving this change. For this purpose, we are able to exploit numerous long term data sets available from the project partners, including on fish stocks, plankton and benthos organisms as well as abiotic environmental conditions. Data series are extended and expanded through a network of Baltic cruises with the research vessels linked to the consortium, and complemented by extensive experimental, laboratory, and modeling work.

From science to management

The ultimate BIO-C3 goal is to use understanding of what happened in the past to predict what will happen in the future, under different climate projections and management scenarios: essential information for resource managers and politicians to decide on the course of actions to maintain and improve the biodiversity status of the Baltic Sea for future generations.

Content

I) Executive Summary of BIO-C3 Task 4.1

The overall **purpose of Task 4.1 was to assess how biodiversity** (species, communities, traits) **respond to (past) abiotic variables** and **how the relationships between biodiversity and ecosystem functioning in the Baltic Sea may have changed over time and/or in response to environmental change over time** (from species to their functions to the community-level functioning).

We have accomplished our goals by showing that long-term changes can be quantified already at the lowest level, the primary producers (work 1, 2, 3 and 9 in *iv)*) throughout the trophic network up to fish (work 7,8,9,10 in *iv)*). However, the signals may vary depending on season, and for example we show that phytoplankton blooms earlier in spring, by 1-2 weeks over the last 20 years, but total summer phytoplankton biomass variation over time, across the Baltic Sea, could not be well-explained by a common temporal trend among stations with little additional explanatory power from regional climate variables. We have targeted both the pelagic and the benthic food chain and its functioning by investigating temporal changes in pelagically feeding fish (work 7 in *iv)*) and long-term changes in community and populations of zooplankton (work 4, 5 in *iv)*) as well as functional changes in coastal benthic macrofauna and fish (work 6, 9 in *iv)*) and the offshore benthic feeding fish community (work 8 in *iv)*). We have related these changes to the natural salinity gradient (work 8, 14 in *iv)*) as well as large scale anthropogenic and climatic drivers (work 2,5,9 in in *iv)*). Concerning the temporal aspects, many studies have been able to capture the suggested regime shift periods in the Baltic Sea (1980s-90s) or investigate the resulting products of it.

Our intention within this task, as outlined in the plan, was to bring in functional traits and trait-based approaches as a novel tool to study and understand changes in ecosystem structure and function. We have done this successfully by evaluating long-term trends and changes in benthic macrofauna (work 6 in *iv)*) where we show that functional identity of communities is as essential to investigate as more common functional measures such as functional richness. We also include functional trait measures to elucidate that long-term functional changes of coastal macorfauna and fish, is showing trends rather than abrupt shifts despite turnover of taxa (work 10 in *iv)*). Trait-based measures were also applied to investigate the community assembly of demersal fish, and highlighted an east-west difference in drivers of fish community composition (work 8 in *iv)*). In addition, we also linked characteristics of species to their functioning, for example in order to predict primary production based on macrophyte functional traits (work 4 in *iv)*). Using physiological, life-history, behavioral and morphological information about individuals and species have thus proven to be an essential way forward for understanding functional changes in the Baltic Sea ecosystem and we recommend bringing such knowledge into future advice and management actions for the region. Nevertheless, we have also evaluated the performance of traditional benthic indices over time (work 11, 12, 13 in *iv)*). We show that these measures provide a good understanding of the long-term progression of one of the most macrofaunal data-rich areas in the Baltic Sea, the Polish coast (work 11 in *iv)*), and a fair response to both eutrophication and sediment dumping pressure (work 12, 13 in *iv)*), althoguh the assessment (particularly, set-up of the sensitivity values) should be adjusted for the ecosystem in question and be habitat-specific. Furthermore we compared the performance of six alternative modelling approaches for obtaining reliable spatial information about fish diversity indices (work 14 in *iv)*). Within Task 4.1 we also evaluated the functional response of non-indigenous species on the benthic community over time (work 6 in *iv)*) and for the performance of benthic indices (12 in *iv)*).

Accurate assessments of ecological changes that have occurred in the past is a prerequisite for predictive analysis and rely generally, as for our studies in Task 4.1, on national and/or regionally gathered monitoring data. Therefore, we further recommend to stakeholders such as HELCOM, ICES, as well as national and regional environmental bodies to make sure that coordination of sampling programs across trophic levels are in place. The difficulty in finding comparable data sets with several trophic or organismal groups sampled within an ecologically relevant area (i.e. where interactions can take place) was evident in many activities within this task.

Our results and knowledge gained so far directly link to other tasks within BIO-C3, especially Task 4.2 and 4.3 (changes in food web and future predictions) as well as WP5 (the role of indicators). Future steps include the realizations of these links and transfer of our gained knowledge, but also a joint publication summarizing the outcomes within this task, planned to be ready for submission within the final year of the project.

II) Introduction

The overarching goal of task 4.1, to identify and quantify changes in, and drivers of change in biodiversity and functioning, it is central for the entire WP4. Understanding past changes in the Baltic Sea ecosystem is pivotal in order to predict any future ones, may it be within the food web (Task 4.2) or future scenarios (Task 4.3). The nature of the task involved synthesis and harmonisation of long-term data from various sources, rather than collection/production of new data through experiments or fieldwork. The task has succeeded in investigating each trophic level or organismal group (from the primary producers, namely phytoplankton and macrophytes, to the consumer levels benthos and fish) in respect to the aims. The geographical coverage was broad and for some organismal groups a retrospective analysis on a Baltic Sea-wide or basin-wide scale have been conducted, while for others, more local or regional scale studies, serving as model systems, have been done.

In total, 14 individual contributions and two datasets were included in this summary report.

III) Core Activity

Task 4.1 aimed at summarising changes in biodiversity indicators (species, communities, indices) across all trophic groups in all parts of the Baltic Sea (coastal as well as offshore), but focused specifically on the following areas for respective organismal group:

- **Phytoplankton** spring-blooming trends were studied in the Baltic Proper, while summer biomass was studied in the whole of the Baltic Sea.

- **Macrophytes** were studied in five coastal areas in the Northern Baltic.

- **Zooplankton** long-term trends were assessed in the South eastern Baltic Sea (Puck Bay and Gulf of Gdansk), while copepods (*E. affinis* and *Acartia* spp.) were specifically investigated in Pärnu Bay in the northeast Gulf of Riga, representing a shallow coastal bay.

- **Macrozoobenthic** community changes over time were investigated in a coastal system (Åland Islands) as well as along the geographical gradient in the Baltic Sea (three coastal areas from Kattegat/Skagerrak to Bothnian Sea). In addition, temporal changes of the benthic community were also analysed in the Bothnian Bay in respect to food web and ecosystem-

level changes. The Polish part of the Baltic Sea as well as the South eastern part of the Baltic Sea (the Lithuanian coast and the Curonian Lagoon) served as model systems for the evaluation of macorfauna-based indices for management purposes.

- **Fish** feeding-patterns with respect to pelagic fish (herring and three-spined stickleback) were studied in Gulf of Riga. Fish community assembly in relation to abiotic drivers was assessed in an area from the Kattegat to the Northern Baltic Proper, while fish community progression (taxonomic and functional) was assessed along the Baltic Sea gradient in three coastal areas (Skagerrak/Kattegat, Baltic Proper and Bothnian Sea). Changes in the herring population in respect to changes in the overall ecosystem (food web) were also assessed in the Bothnian Bay.

The following major activities were conducted in Task 4.1, specified per contributing partner:

P2 examined patterns and drivers of fish community assembly of demersal fish in the Kattegat and Baltic Proper (joint work with P13).

P4 assessed long-term phytoplankton spring bloom data as well as summer phytoplankton biomass in relation to climate and environmental factors.

P5 analysed long-term trends in zooplankton and evaluated the performance (temporal variability) of macrofaunal quality indices as well the performance of selected machine learning and regression-based methods for modelling fish community indices.

P6 investigated copepod inter-annual variability and seasonality, assessed feeding trends of pelagic fish from stomach data. In relation to these actions, metadata have also been synthesized and published (for details see Appendix 15 and 16). P6 also investigated macrofaunal trait data in relation to primary production.

P7 summarized and assessed long-term changes of the Bothnian Bay ecosystem on multiple trophic levels.

P8 evaluated the benthic quality index (BQI) to eutrophication and physical disturbance over time.

P13 investigated long-term benthic community and functional diversity trends in the Åland Islands in relation to invasive species and abiotic environmental drivers for a 40 year period, and assessed long-term functional development of coastal macrofauna and fish along the Baltic Sea gradient, as well as drivers of fish community assembly (joint work with P2).

IV) Scientific Highlights

Retrospective analysis of species, communities or trophic groups in relation to ecosystem functioning

1. Climate driven changes in timing, composition and size of the Baltic Sea phytoplankton spring bloom
(for details see Appendix 1)

Conspicuous spring phytoplankton blooms provide food for pelagic grazers and benthos and influence biogeochemical cycles in many temperate aquatic systems. Understanding environmental effects on e.g. spring bloom dynamics is important for predicting future climate responses and for managing aquatic systems. We analyzed long-term phytoplankton data from one coastal and one offshore station in the northwestern Baltic Proper to uncover trends in timing, composition and size of the spring bloom and its correlations to environmental variables. There was a general trend of earlier blooms by 1-2 weeks over the last 20 years, correlated to more sunshine and less windy conditions. High water temperatures were correlated to earlier blooms of diatoms and dinoflagellates that dominate the spring bloom. Overall bloom timing, however, was buffered by a temperature and ice related shift in composition from early blooming diatoms to later blooming dinoflagellates and *Mesodinium rubrum.* Such counteracting responses to climate change highlight the importance of both general and taxon-specific investigations. We hypothesized that the effect of the predicted temperature increase on the timing of spring blooms in the Baltic Sea might be counteracted by more clouds and stronger winds. A shift from early blooming and fast sedimenting diatoms to later blooming groups, alongside higher temperatures is expected to increase energy transfers to pelagic secondary production and decrease spring bloom inputs to the benthic system, resulting in lower benthic production and lower oxygen consumption.

2. Limited evidence for common multi-decadal trends in Baltic Sea summer phytoplankton biomass
(for details see Appendix 2)

Identifying the scales at which phytoplankton dynamics operate is crucial to evaluating the effect of these dynamics on aquatic ecosystem functions. The Baltic Sea summer phytoplankton community plays an important role in biogeochemical cycling and in the transfer of energy through the food web via zooplankton. We used dynamic factor analysis to study if there are temporal common trends in multi-decadal summer phytoplankton total and class-level biomass time series across Baltic Sea latitudinal gradients in salinity and temperature. We hypothesize that summer phytoplankton biomass throughout the Baltic Sea would exhibit common low-frequency temporal variation structured by both geography and taxonomic identity. Total summer phytoplankton biomass variation over time across the Baltic Sea could not be well-explained by a common temporal trend among stations with little additional explanatory power from regional climate variables. For major phytoplankton taxonomic groups (classes) that regularly contribute to summer biomass, we do find some evidence that common temporal trends can explain their variance over time. Common trends are most closely associated with cryptophyte time series across the Baltic Sea and are particularly sensitive to the synchronous disappearance of cryptophytes in two years. The trend loadings of cyanobacteria, a major component of the summer community, were variable and without geographic coherence. The Baltic Sea Index is correlated with two common class trends, both showing shifts in mean state around the mid-1990s. There was only limited

evidence for common temporal trends among class time series and stations if the two years with absent cryptophytes were removed. The limited coherence in the local dynamics of phytoplankton communities over time throughout this large heterogeneous ecosystem suggest that broad generalizations are limited and the emergent ecosystem dynamics maybe bear little resemblance to the localized dynamics of communities.

3. Functional traits of marine macrophytes predict primary production
(for details see Appendix 3)

Large seaweeds and higher plants (macrophytes) are commonly found in coastal marine habitats where they photosynthesise i.e. they build up their own organic compounds by using the sun's energy. Primary production underpins aquatic food webs, plays an important role in the global carbon cycle and connects the life from surface ocean layers with the species at the seabed. However, the rate of primary production can greatly vary between species, communities, ecosystems, seas and oceans, simply because species differ in terms of their functional setup and oceans in their environmental settings. Here we investigated the possibility to link functional traits (i.e. biological characteristics) of macrophytes from the major European seas to primary production and we asked if there were specific functional traits that could be effectively used to predict primary production of macrophyte communities. We first measured primary production of macrophyte communities and then quantified how large fractions of the total community biomass different traits compose. By linking community production and trait information we found out that primary production mostly depended on the amount of large epilithic seaweeds of marine origin in the community. Interestingly, we also found that several traits were clustered together meaning that the occurrence of one trait increased the likelihood of another. Our findings suggest that functional traits of macrophytes can be effectively used to monitor primary production. Furthermore, taxonomically distinct species might possess similar traits meaning that functional aspects of ecosystems can be analysed without extensive taxonomic knowledge. This could result in simplification of the general procedure of production estimations and establish transparent framework how to link community structure with functioning.

4. Long-term changes in zooplankton community structure in the southern Baltic Sea
(for details see Appendix 4)

Mesozooplankton is a key element in marine food web. In the southern Baltic Sea conditions, it is extremely vulnerable to environmental forcing. The availability of zooplankton prey is variable and considerable changes in the species composition have been suggested as a result of changes in the deep water salinity (decline in *Pseudocalanus spp*. since the 1980s), temperature (increase in *Temora longicornis* and *Acartia spp*. during the 1990s), and predation pressure. Thus, differences in abundance of key zooplankton species was analyzed based on long-term monitoring data collected in the southern Baltic Sea. For the purpose of this preliminary analysis, data from the deep-water stations located in the Bornholm Basin (P5), Gdansk Basin (P1) and at the southern slope of the Gotland Basin (P140) as well as from the very coastal station ZP6 located in the Puck Bay were selected. To verify if there are any temporal trends in the dominance of copepods and cladocerans in the total zooplankton abundance, we used data from three stations with decreasing distance to the coastline located in the Gulf of Gdansk. Profound changes in zooplankton community were recorded at the deep-water stations of the southern Baltic Sea. This is mostly caused by a decrease in abundance of *Pseudocalanus* copepods. *Pseudocalanus* is correlated with salinity and, thus, positively responding to each of the inflows of saline water masses from the North Sea.Similar changes were not observed in more shallow-water stations but those data series

are still not long enough for detailed analyses as sampling there started at the beginning of the 21st century. No clear long-term patterns were detected for *Acartia* and *Temora* copepods and further analyses are necessary to identify factors responsible for the observed spatial and temporal dynamics.

5. Winter-spring climate effects on small copepods in the shallow coastal sea
(for details see Appendix 5, for data see Appendix 15)

Winter severity and hydroclimate in winter-spring period affects spring-summer zooplankton dynamics in the northern hemisphere coastal seas. We analysed the seasonal dynamics, abundance and population age composition of small copepods, Eurytemora affinis and *Acartia* spp. in a shallow coastal habitat in the Baltic Sea during the spring-summer period (May-July) of 1957-2012, in response to winter severity, and monthly mean sea surface temperature (SST). Three proxies for winter severity (Baltic Sea Index, locally measured winter air temperature, ice break off day) were tested, winter air temperature in combination with SST best predicted the abundances of both taxa. Ambient SST has increased in the study area, despite of negative trend in the winter air temperature since 1990. E. affinis seems to suffer higher mortality during development, has a stronger seasonal pattern, and more variable phenology than *Acartia* spp. Universally, both taxa, and all development stages were more abundant in May and June following milder winters. Monthly mean SST affected most the adults, implying reduced mortality during development from juveniles to adults at elevated ambient temperature. Hence, the climate effect on the secondary producers in shallow high latitude seas acts through multiple and partly independent mechanisms, since the seasonal dynamics and abundance were linked to winter severity, and the population structure to ambient SST. Lack of quantitative long-term information on all predators hampers incorporation of food-web interactions and therefore limits the power of conclusions.

6. Maintained functional diversity in benthic communities in spite of diverging functional identities
(for details see Appendix 6)

We studied zoobenthic communities of two habitats, sheltered and exposed, of a coastal system subject to contrasting changes in community composition over the past four decades. Besides eutrophication and climate-related impacts, the system has been invaded by a non-native polycheate, *Marenzelleria* spp., adding altered functional properties to the communities. The functional dispersion (FDis) metric was used as a measure for comparing the functional diversity of the contrasting habitats, with special focus on the role of *Marenzelleria* for the entire communities. We highlight changes in the functional identity of the communities, expressed as community-weighted means of trait expression (CWM), using multivariate techniques, and investigate the relationship between taxonomic and functional changes. Despite contrasting community developments in the two habitats, with characteristics traditionally suggesting different environmental quality, we found that the FDis in both habitats remained similar and increased with the introduction of *Marenzelleria*. Although showing maintained functional diversity across time and space, the functional identity (CWM) of communities changed irrespective of taxonomical differences. Examples include inter alia alterations in palatability proxies, feeding position and sediment transportation types, indicating changed functionality of zoobenthos in coastal systems. We show, when focusing on qualitative functional changes of communities, it is important to evaluate the underlying functional identity, and not only rely on measures of the diversity of

functions per se, as the quality indication of expressed functional traits can be concealed when using multi-functionality approaches.

7. Feeding patterns of small pelagic fish in Gulf of Riga
(for details see Appendix 7, for data see Appendix 16)

We investigated the feeding of the dominant small pelagic fish - herring *Clupea harengus membras* and three-spined stickleback *Gasterosteus aculeatus* - in the Gulf of Riga (Baltic Sea) in the summers of 1999-2014. The share of empty stomachs, stomach fullness and taxonomic composition of fish diet was analysed. On average, large herring had the highest (19%) and small herring the lowest (6%) share of empty stomachs. Small (< 1mm) cladoceran *Bosmina* spp. was most the important prey for three-spined stickleback; preying on small (<1.5 mm) copepod Eurytemora affinis was most efficient for small herring, while *Bosmina* spp. and E. affinis were equally important for the large herring, followed by the large (mean body lenght < 2.0 mm) non- indigenous cladoceran *Cercopagis pengoi*. The number of prey taxa per stomach exhibited significant differences between the fish groups studied, the highest mean value was recorded for small herring and the lowest for three-spined stickleback (2.1 and 1.4 taxa, respectively). Though present, the fish-group specific spatial dynamics in feeding parametres (share of empty stomachs and feeding intensity) were weak compared to the observed inter-annual variation.

8. Patterns and drivers of fish community assembly in a large marine ecosystem
(for details see Appendix 8)

The presence and survival of the species in a community depend on their abilities to maximize fitness in a given environment. The study of the processes that control survival and co-existence, termed ‘assembly rules’, follows various mechanisms, primarily related to biotic or abiotic factors. To determine assembly rules, ecological similarities of co-occurring species are often investigated. This can be evaluated using trait-based indices summarizing the species’ niches in a given community. In order to investigate the underlying processes shaping community assembly in marine ecosystems, we investigated the patterns and drivers of fish community com- position in the Baltic Sea, a semi-enclosed sea characterized by a pronounced environmental gradient. Our results showed a marked decline in species- and functional richness, largely explained by decreasing salinities. In addition, habitat complexity and oxygen were found to be significant drivers. Furthermore, we showed that the trait composition of the fish community in the western Baltic Sea is more similar than expected by random chance alone. This implies that environmental filtering, acting along the salinity gradient, is the dominant factor shaping community composition. However, community composition in the eastern part, an area beyond the steep decline in salinity, was characterized by fewer species with largely different trait characteristics, indicating that community assembly is also affected by biotic interactions. Our results add to the knowledge base of key abiotic drivers impacting marine fish communities and their vulnerability to environmental changes, a key concern for fisheries and marine ecosystem management.

9. A retrospective view on the development of the Gulf of Bothnia ecosystem
(for details see Appendix 9)

We analysed long-term monitoring data from 1979 to 2012 in the Gulf of Bothnia. Variation in deep-water intrusion from the main Baltic Proper had a clear effect on the phyto- and zooplankton communities in the Bothnian Sea. The nutrient status in this basin has also changed towards nitrogen limitation with subsequent class- and genus-level changes in

phytoplankton community composition. The migration of cod to the Bothnian Sea during the 1980s had profound effects on the herring population, but cascading effects affecting the basis of the food web were not obvious. Results point to a deteriorating eutrophication trend in the Bothnian Sea.

10. Long-term functional trends of coastal zoobenthos and fish in the Baltic Sea
(for details see Appendix 10)

Our understanding of changes in biodiversity has greatly improved by shifting the focus from primarily species richness as the measure of diversity to functional characteristics and the identity of species. We still lack information on the temporal variability of such functional measures as well as the interlinkage between several trophic groups. In this study, we investigated the long-term temporal (~ 40 years) pattern in functional structure of fish and benthic macrofauna in three coastal areas with different environmental conditions located in Kattegat, the Baltic Proper and the Bothnian Sea. Building on previous long-term studies on changes in taxonomic structure, we assembled trait information on six traits spanning morphology, life history and behaviour for over 100 macrofaunal taxa and six traits describing diet, habitat and productivity for over 52 fish taxa. To assess functional changes we analysed key functional indices (FRic, FEve and FDis) as well as functional composition (CWMs) and showed that functional changes in the different areas and trophic groups are gradual and stepwise, rather than abrupt shifts. Timing of changes differed between areas and between trophic groups within the same area, highlighting the different histories in potential drivers of change (eutrophication, climate and fishing pressure). Functional indices showed in general an increasing trend within the Baltic Sea and development in the functional composition over time was similar for both macrofauna and fish. In Kattegat, a decreasing trend was identified, with functional richness responding the strongest. These differences between areas in functional trends are valuable information in a management perspective and we urge the incorporation of functional properties (both indices and composition) into management practices and environmental advice.

Retrospective analysis of Indices

11. Performance of macrozoobenthos diversity indices: Benthic Quality Index (BQI), Shannon-Wiener diversity (H), Margalef diversity (d).
(for details see Appendix 11)

The study was aimed at determining long-term variability in benthic communities in the Polish Marine Areas by analysing values of biocoenotic indices calculated for different benthic invertebrate communities studied since 1980. Data collected within the framework of different projects carried out by the National Marine Fisheries Research Institute in 1978-2015 were used. Applicability of older available quantitative data (collected since 1935) was analysed as well. Analyses of diversity indices for the macrozoobenthos inhabiting the Polish part of the Baltic Sea allowed identifying patterns in long-term variability. An emergent characteristic is the long-term stability of macrobenthic communities occurring at depths shallower than about 60- 70 m, and even down to 90 m in the Słupsk Furrow. Unfavourable changes in the community structure, and even a complete disappearance of the fauna, on the muddy bottom of the southern part of the Bornholm Deep and the Gulf of Gdańsk, also above the halocline, are related to oxygen depletion in those parts of the Baltic Sea. The disappearance of the macrobenthos in deeper areas of the Polish part of the Baltic Sea is most likely a factor affecting food availability for fish. More studies are required to assess the

importance of this factor. A reliable assessment of the present status of the macrobenthos calls for collection of additional data, primarily from the sandy bottom of the open sea, down to the depth of about 70 m.

12. Benthic Quality Index (BQI) response to invasive species (physical disturbance)
(for details see Appendix 12)

Benthic component of an ecosystem is considered in ecological status assessment of the key European Directives. Most of the metrics proposed for the benthic quality assessment are biodiversity based. Their robustness and applicability are widely discussed in many recent studies. However, an impact of invasive alien species on biotic indices and environmental quality assessments has been largely overlooked by researchers so far. We assessed Benthic Quality Index (BQI) in a coastal ecosystem, highly affected by the invasive zebra mussel *Dreissena polymorpha*. Zebra mussel is able of modifying benthic habitats and enhancing local biodiversity. In the analyzed ecosystem it affected benthic species richness, abundance and community structure. As a result, the calculated BQI values were significantly higher in the presence of zebra mussel with evident outliers in samples with particularly high zebra mussel abundances. Therefore, we found that BQI determined in our study was artificially elevated providing false signal of the ecological status improvement. Based on the results presented, we suggested data correction framework that has been tested on the current dataset and proved to be effective minimizing zebra mussel impact on BQI assessment. Our experience could be applied for other coastal ecosystems invaded by the zebra mussel or any other aquatic invasive species with resembling biological traits and bio-invasion impacts.

13. Performance of the Benthic Quality Index (BQI) to eutrophication in the SE Baltic Sea
(for details see Appendices 13)

The European Marine Strategy Framework Directive requires EU Member States to prepare national strategies and manage their seas to achieve good environmental status (GES) by 2020. There are many multimetric indices proposed as indicators of the ecological quality of the benthic environment. Their functionality and utility are extensively discussed in the literature. Different frameworks are suggested for comparative assessments of indicators with no agreement on a standardized way of selecting the most appropriate one. In the current study, we apply signal detection theory (SDT) to evaluate the specificity and sensitivity of the Benthic Quality Index (BQI), its response to the eutrophication pressure, and its performance under the effects of estuarine water outflow. The BQI showed acceptable response to total nitrogen, total phosphorus and chlorophyll-a concentrations in the study area. Based on the results, we suggest using SDT for setting GES thresholds in a standardized way. This aids a robust assessment of the environmental status and supports differentiation between the quality classes.

14. Spatial prediction of demersal fish diversity in the Baltic Sea: comparison of machine learning and regression-based techniques
(for details see Appendix 14)

Marine spatial planning (MSP) is considered a valuable tool in the ecosystem-based management of marine areas. Predictive modelling may be applied in the MSP framework to obtain spatially explicit information about biodiversity patterns. The growing number of statistical approaches used for this purpose implies the urgent need for comparisons between

different predictive techniques. In this study, we evaluated the performance of selected machine learning and regression-based methods that were applied for modelling fish community indices. We hypothesized that habitat features can influence fish assemblage and investigated the effect of environmental gradients on demersal fish diversity (species richness and Shannon–Weaver Index). We used fish data from the Baltic International Trawl Surveys (2001–2014) and maps of six potential predictors: bottom salinity, depth, seabed slope, growth season bottom temperature, seabed sediments and annual mean bot- tom current velocity. We compared the performance of six alternative modelling approaches: generalized linear models, generalized additive models, multivariate adaptive regression splines, support vector machines, boosted regression trees and random forests. We applied repeated 10-fold cross-validation, using accuracy as the measure of model quality. Finally, we selected random forest as the best performing algorithm and implemented it for the spatial prediction of fish diversity from the Baltic Proper to the Kattegat. To obtain information on the data reliability and confidence of the developed models, which are essential for MSP, we estimated the uncertainty of predictions with standard deviation of predictions obtained from all the trees in the ensemble random forest method. We showed how state-of-the-art predictive techniques, based on easily available data and simple Geographic Information System tools, can be used to obtain reliable spatial information about fish diversity. Our comparative work highlighted the potential of machine learning method to reduce prediction error in modelling of demersal fish diversity in the framework of MSP.

V) Progress and next steps

Studies and activities were performed according to the workplan and original objectives were obtained, except for the task on retro-statistical analysis on linkages between cod and benthic prey, which is still in progress (*see below for details and justifications*).

Out of the 14 studies included in this deliverable, five are published, three are in press, three are under peer-review, and three are being prepared for submission.

Work is still ongoing regarding the planned study on cod-benthic prey linkage (P2). This manuscript in preparation has progressed towards establishing a database of abundance of the isopod, *Saduria entomon*, from various sources around the Baltic Sea. Data available in the HELCOM monitoring database now held at ICES, and data maintained at IOW are being merged, and ensuring that duplicate observations are eliminated. The formats of the data are somewhat heterogeneous and in some cases initially prevented abundance estimation. For example, initial requests for HELCOM data from ICES resulted in provision of datasets with only the stations where the species was captured, but excluding zero-catch stations. Furthermore, the biological data needed to be merged with bathymetric and other station-specific data, which was not always available for all records. The database has therefore taken more time to prepare than anticipated, but a preliminary presentation of some of the data was made at the ICES Workshop on Workshop on Spatial Analyses for the Baltic Sea (WKSPATIAL) in November 2015. We now have a version that will form the basis of new analyses in the coming months of trends and variability in abundance and spatial distribution, and how these variations could affect cod ecology. This sub-task will be reported in an upcoming annual report.

Concerning the transfer of outcomes to other work packages and tasks, the results gained in task 4.1 are central to the other tasks within WP4, and much of the results are valuable for and feed into specifically Task 4.3 (predicting future changes). The results on e.g. the usefulness

of functional measures and performances of benthic indices (BQI etc.) for identifying changes in the Baltic Sea ecosystem are also valuable for WP5 (use of potential indicators). Task 4.1. has in addition gained input from Task 1.3 and 2.1 in respect to cod historical patterns and changes and Task 2.2 regarding top-down control on *Saduria* and time-series on phytoplankton. In addition, knowledge gained regarding historical development of cod, flounder, *Pseudocalanus* sp. and pH within Task 3.2 have been of use for the activities in Task 4.1. as well. The results showing how the functional properties of the ecosystem adapt and develop in response to environmental change, are of key-importance for future management-scenarios.

Furthermore, a joint publication, with all partners within task 4.1, summarising the past changes in different trophic groups (with either or both indices, species and communities) in the Baltic Sea is planned for 2017 and will be an additional major outcome of this task.

All future outcomes of task 4.1. will be included in forthcoming reports.

VI) Methods and results

Major results are highlighted in section (i), detailed methods and results for each respective study are described in the Appendices in section (VIII)

VII) Recommendations

Based on the outcomes and conducted work within this task, we i) **recommend the inclusion of functional properties into national and regional advice** (e.g. HELCOM, ICES). The knowledge-gains and the usefulness of e.g. trait-based approaches and functional measures is a way forward in addition to the use of traditional numerical indices, as the overall analysis gets beyond species-identities. The work presented in this report provides excellent examples of this (e.g. Appendices 3,6,8 and 10). As the applicability of such methods and measures are taxa transcending, they also provide possibilities for studying the ecosystem and the food webs together. However, this also requires availability of data (e.g. density, biomass) across trophic levels, which is our second recommendation (see below). Within many of the activities striving to include several trophic levels or organismal groups, data availability was a challenge, although the Baltic Sea is one of the world's most studied sea areas. For example, within the study on long-term functional changes in coastal benthos and fish, data were only available from the western part of the Baltic Sea since sampling programs for the two organism-groups (zoobenthos, fish) are not overlapping in other areas. Therefore, we **ii) recommend and call for more coordinated monitoring activities across trophic levels in time and space**. It is essential that all trophic levels are sampled in the same place (within an ecologically meaningful area) continuously and with a long-term perspective. We see a strong potential for stakeholders such as HELCOM and ICES to facilitate such actions.

VIII) Appendices

APPENDIX 1: Climate driven changes in timing, composition and size of the Baltic Sea phytoplankton spring bloom (manuscript under peer-review)

APPENDIX 2: Limited evidence for common multi-decadal trends in Baltic Sea summer phytoplankton biomass (manuscript under peer-review)

APPENDIX 3: Functional traits of marine macrophytes predict primary production (article in press in *Functional Ecology*, published online 2016)

APPENDIX 4: Long-term changes in zooplankton community structure in the southern Baltic Sea (manuscript in preparation for submission)

APPENDIX 5: Winter-spring climate effects on small copepods in the shallow coastal sea (manuscript under peer-review)

APPENDIX6: Maintained functional diversity in benthic communities in spite of diverging functional identities (article published in OIKOS 2016)

APPENDIX 7: Feeding patterns of small pelagic fish in Gulf of Riga (article in press in *Hydrobiologia*, published online 2016)

APPENDIX 8: Patterns and drivers of fish community assembly in a large marine ecosystem (article published in Mar. Ecol. Prog. Ser. 2016)

APPENDIX 9: A retrospective view on the development of the Gulf of Bothnia ecosystem (article in press in *Journal of Marine Systems*, published online 2016)

APPENDIX 10: Long-term functional trends of coastal zoobenthos and fish in the Baltic Sea (manuscript in preparation for submission)

APPENDIX 11: Performance of macrozoobenthos diversity indices: Benthic Quality Index (BQI), Shannon-Wiener diversity (H), Margalef diversity (d). (manuscript in preparation for submission)

APPENDIX 12: Invasive ecosystem engineers and biotic indices: Giving a wrong impression of water quality improvement? (article published in Ecological Indicators 2015)

APPENDIX 13: Application of signal detection theory approach for setting thresholds in benthic quality assessments (article published in Ecological Indicators 2016)

APPENDIX 14: Spatial prediction of demersal fish diversity in the Baltic Sea: comparison of machine learning and regression-based techniques (article published in ICES Journal of Marine Science 2016)

APPENDIX 15: Metadata sheet: Baltic Sea mesozooplankton dataset (Joint dataset with EU BONUS project INSPIRE)

APPENDIX 16: Metadata sheet: Herring and three-spined stickleback feeding in the Gulf of Riga (Joint dataset with EU BONUS project INSPIRE)

Appendix 1.

This manuscript is under embargo until it has been published. If you are interested in the results or data, please contact the corresponding author:

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Appendix 2.

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Appendix 3.

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ABSTRACT:

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Functional traits of marine macrophytes predict primary production

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Summary

1. The relationship between community structure and the functioning of ecosystems is the sub- ject of ongoing debate. Biological or functional trait-based approaches that capture life strat- egy, morphology and behavioural characteristics have received far less attention than taxonomic diversity in this context, despite their more intuitive link to ecosystem functioning.

2. Macrophyte primary production underpins aquatic food webs, regulates benthic and pelagic ecosystems and is a key aspect of the global carbon cycle. This study spans a range of aquatic biomes across Europe and aims to examine potential for predicting primary production of macrophyte communities based on the functional traits of species and identify the traits that are the most informative indicators of macrophyte production.

3. Macrophyte primary production was assessed based on the oxygen production of the whole community, linked to biomasses of selected biological traits derived of its component species and analysed using the novel boosted regression trees modelling technique.

4. Results showed that functional traits derived from macrophyte community data explained most of the variation in primary production of macrophyte communities

without the need to incorporate environmental data on the habitats. Macrophyte primary production was influ- enced by a combination of tolerance, morphology and life habit traits; however tolerance traits contributed most of variability in macrophyte primary production when all traits were anal- ysed jointly.

5. This study also showed the existence of trait clustering as the studied trait categories were not fully independent; strong interlinkages between and within trait categories emerged.

6. Our study suggests that functional trait analysis captures diðerent aspects of ecosystem functioning and thereby enables assessing primary production of macrophyte communities over geographically distinct areas without extensive taxonomic and environmental data. This could result in a novel framework through which a simplification of the general procedure of production estimations and comparisons across environmental gradients can be achieved.

Key-words: algae, benthic communities, biological traits, community ecology, community production, ecosystem ecology, ecosystem functioning, functional characteristics

Appendix 4.

This manuscript is under embargo until it has been published. If you are interested in the results or data, please contact the corresponding author:

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Appendix 6.

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Maintained functional diversity in benthic communities in spite of diverging functional identities

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Ecological studies based on time-series often investigate community changes centered on species abundance or biomass but rarely expose the consequential functional aspects underlying such changes. Functional diversity measures have proven to be more accurate predictors for ecosystem functioning than traditional taxonomic approaches and hence gained much attention. There are only limited studies available that analyse the functional implications behind decadal changes of entire communities. We studied zoobenthic communities of two habitats, sheltered and exposed, of a coastal system subject to contrasting changes in community composition over the past four decades. Besides eutrophication and climate-related impacts, the system has been invaded by a non-native polycheate *Marenzelleria* spp., adding altered functional properties to the communities. The functional dispersion (FDis) metric was used as a measure for comparing the functional diversity of the contrasting habitats, with special focus on the role of *Marenzelleria* for the entire communities. We highlight changes in the functional identity of the communities, expressed as community-weighted means of trait expression (CWM), using multivariate techniques, and investigate the relationship between taxonomic and functional changes. Despite contrasting community developments in the two habitats, with characteristics traditionally suggesting different environmental quality, we found that the FDis in both habitats remained similar and increased with the introduction of *Marenzelleria*. Although showing maintained functional diversity across time and space, the functional identity (CWM) of communities changed irrespective of taxonomical differences. Examples include inter alia alterations in palatability proxies, feeding position and sediment transportation types, indicating changed functionality of zoobenthos in coastal systems. We show, when focussing on qualitative functional changes of communities, it is important to evaluate the underlying functional identity, and not only rely on measures of the diversity of functions per se, as the quality indication of expressed functional traits can be concealed when using multi-functionality approaches.

Changes in environmental conditions, largely forced by anthropogenic and climatic drivers, influence community structures of ecosystems on a global scale (Vitousek 1994, Harley et al. 2006, Doney et al. 2012). Ecological studies investigating those effects over decades often examine community changes based on species abundance, biomass or diversity but rarely expose the consequential functional progression of such changes. Using ecological functionality of species, i.e. the expression of their functional traits, rather than their taxonomic identity, has gained much attention in the field of ecology. Trait-based approaches promise better understanding of the relationships between organisms and ecosystem functioning (Gagic et al. 2015). Several studies have pointed out that the functional diversity within a community may act as key driver in explaining the magnitude of ecosystem processes (Tilman et al. 1997, Solan et al. 2004), the resilience towards changing environmental conditions (Folke et al. 2004) and the range of ecosystem services in general (de Bello et al. 2010). Recognizing the ecological relevance derived from functional diversity measures, much work has been done to develop indices being able to capture it (Petchey and Gaston 2002, Villéger et al. 2008, Laliberté and Legendre 2010), including the comparison and evaluation of such indices (Lavorel et al. 2008, Schleuter et al. 2010, Mason et al. 2013). Most of the work has been conducted and established on terrestrial plant communities while comparably few studies implemented this approach on faunal communities. Functional diversity is sometimes used as the sole explanatory variable for ecosystem functioning, which on the one hand has been shown to be a stronger predictor than species richness in many circumstances, but on the other omits information on the actual underlying functional structure (the functional identity), leaving it open which functions may or may not be present and to which degree they are expressed (Gagic et al. 2015). There are surprisingly few studies available where the traditional taxonomic approach of changing faunal communities is assessed in combination with a functional framework on empirically collected data (van der Linden et al. 2012, Clare et al. 2015). This is however an asset for understanding the potentially changing functionality within a system along environmental change. Coastal zones can be considered particularly well

suited for such approaches as they represent highly productive ecosystems, where long-term changes due to anthropogenic or environmental drivers are manifested in the mainly stationary and relatively long-lived zoobenthos. Benthic fauna are key actors in a multitude of ecosystem processes and a paramount food resource for benthic predators, especially in coastal zones (Greenstreet et al. 1997, Nilsen et al. 2006, Snickars et al. 2015). Traditionally, taxonomic identities and biomasses of zoobenthos are considered as quality proxy for marine ecosystem health and functioning (Pearson and Rosenberg 1978, Perus et al. 2007). However, the broad spectrum of phenotypic variation among zoobenthos species, including their size range, energy content, protection or environmental position can influence the quality of their ecosystem service. Not every species displays the same range of palatability from a fish-food perspective, nor does every species rework the sediment in similar ways or depths.

We studied a coastal system in the northern Baltic Sea, where long-term changes in zoobenthic communities have been observed over a 40-year time frame with contrasting developments in sheltered and exposed areas, which have been affected by system-specific environmental drivers. Sheltered areas have undergone pronounced changes in species composition in addition to continuously decreasing biomasses, while exposed sites show increasing biomasses and higher overall species richness. These changes have been detailed in Weigel et al. (2015). Furthermore, the studied system also encompasses the large-scale invasion of a non-native species, the polychaete *Marenzelleria* spp., which became highly dominant especially in the exposed areas over the past decade (Weigel et al. 2015). However, the functional role of this new addition to the existing species complex still remains uncertain (Norkko et al. 2012, Kauppi et al. 2015, Maximov et al. 2015).

This background, including a species invasion and the dissimilar progression of the communities over four decades, creates a suitable case study to demonstrate how changing community patterns may also result in altered functional properties, underlining the advantage of including functional trait-based approaches in addition to taxonomy-based approaches. We examine the functional diversity of the sheltered and exposed communities, measured as abundance-weighted functional dispersion (FDis) (Laliberté and Legendre 2010). Further we analyse potential shifts in the functional identity of the community, expressed via changes in the community-weighted means of trait-category expressions (CWM), and investigate if these functional changes follow the taxonomic changes in the communities. With the traits applied we focus on two functional aspects within coastal systems, namely; 1) the quality of zoobenthos as food resource for higher trophic levels, with traits related to production, susceptibility to predation and palatability, including a trait providing species specific energy estimation that has not been used previously; and 2) the role of zoobenthos as ecosystem engineers, including traits related to habitat modification, bioturbation, spatial resource dynamics and elemental cycling. Focussing on these fundamental ecosystem services offers new knowledge regarding the qualitative state of coastal zoobenthos in a system being differentially affected by environmental drivers. We exemplify the usefulness of combining the two functional measures, FDis and CWM, as both reveal complimenting aspects of community functionality. While FDis serves as a proxy for the pure diversity of expressed functional traits, CWM is able to showcase the underlying qualitative features of the present trait expressions and their implications for ecosystem functioning.

This study provides a framework of coupling traditional community-based analytical tools with novel approaches to determine multi-functional changes of communities by highlighting the specific alterations in trait composition and their functional implications for the studied ecosystem.

Material and methods

Study area

Our study area, the Åland archipelago, is located in the northern Baltic Sea at the junction of the Baltic Proper, the Bothnian Sea and the Gulf of Finland (60°15′N; 19°55′ E; see Supplementary material Appendix 1 Fig. A1 for detailed map). This complex seascape comprises a mosaic of different habitats, forming a heterogeneous coastal environment with steep environmental gradients along sheltered bays and exposed shores, reflecting a wide range of coastal types in the Baltic Sea and elsewhere. To study how communities and their functionality changed over time and space, we include 16 stations, with eight located in sheltered and eight in exposed coastal zones. Each station was sampled between late spring and early summer in 1973, 1989, 2000 and 2013. All zoobenthos samples were collected with an Ekman-Birge grab sampler (289 cm^2) taking five replicates per site and directly fixed in a 4% buffered formaldehyde solution. In the lab the organisms were identified to their lowest practical taxonomic level under a stereomicroscope. The applied exposure classification derives from an established exposure index for northern Baltic Sea coasts by Isæus (2004). The exposure-classification offers a habitat proxy for the exposure to waves and wind, depth and the proximity to land, which are important structuring components for biological communities. Hence, sheltered sites are generally shallower, closer to land and less influenced by wind and wave action compared to exposed sites. Further details about the sampling design and exposure groupings can be found in the work from Weigel et al. (2015) who showed that communities of the included sheltered and exposed habitats were clearly distinguishable in terms of species identity, biomass progression and their underlying environmental drivers.

Functional trait selection and data analysis

We developed one novel trait providing a species-specific energy content proxy (kJ g^{-1}), giving an estimation of palatability and quality as food resource (prey) for consumers (predators) in the ecosystem at hand. For the energy estimations we followed established biometric conversion factors from existing literature (Ankar and Elmgren 1976, Rumohr et al. 1987, Brey 2001, Brey et al. 2010). In cases where there were no species-specific data available, we applied the values of the closest taxonomic level. The highest species energy content was set as upper reference level to divide the community into three energy categories, high, medium and low

(Supplementary material Appendix 2 Table A1). In addition we applied eight more functional traits, including 34 trait categories (Table 1), that we selected from the trait catalogue of Törnroos and Bonsdorff (2012).

As some species show plasticity in expression of certain trait categories, we applied the fuzzy coding procedure (Chevenet et al. 1994) with values from 0 to 3, where 0 represents no affinity, and 3 represents full affinity to the expressed category (Törnroos and Bonsdorff 2012). The fuzzy-coded species-trait matrix was then abundance-weighted using the average species abundances (of five samples) for each of the 16 stations within each sampled year.

We consider functional diversity as the diversity in distribution and range of expressed functional traits, with the latter reflecting characteristics of organisms that influence ecosystem processes (Díaz and Cabido 2001, Petchey and Gaston 2006). For assessing the functional diversity and functional identity of communities in sheltered and exposed habitats, we used the 'FD' library (Laliberté et al. 2014) in the open source statistical computing software R ver. 3.0.2 (<www.r-project.org>). We chose the functional dispersion (FDis) metric, developed by Laliberté and Legendre (2010), as diversity index as it has certain analytical advantages in comparison to other indices. The metric is unaffected by species richness, can be used with more traits than species present, can be computed from any distance or similarity measure, and it is capable of taking species abundances into account (Laliberté and Legendre 2010) as was desired in this study. FDis describes the abundance-weighted mean distance of individual species to their group centroid (all species of a community) in a multivariate trait space. Larger FDis values imply a more functionally spread community in the multivariate trait space, and hence a higher functional diversity. The tighter species are clustered around their group centroid, the less functionally diverse is the community and the smaller is FDis. Because of the massive invasion by the non-native polychaete *Marenzelleria* in the early 1990s and its established dominance in our study system, we further examined if the addition of *Marenzelleria* has an effect on FDis in the respective habitats. For this we additionally calculated the hypothetical FDis for present communities with the exclusion of *Marenzelleria*. To test if FDis followed the contrasting development of zoobenthic communities between the exposure groups and over time, shown by Weigel et al. (2015), we applied a linear mixed-effect model, with exposure and year as fixed factors and

Table 1. List of included functional traits (9), trait categories (37), and functions. Listed labels correspond to trait categories in Fig. 3, Supplementary material Appendix 3 Fig. A3.1, A3.2.

Trait	Category		Labels	Function
Maximum size	Small	1–5 mm	S	
	Medium	5–30 mm	M	Proxy for ecosystem functioning and palatability
	Large	>30 mm	L	
Protection	No protection		npr	Proxy for palatability (production)
	Tube		tub	
	Burrow		bur	
	Case		cas	
	Soft shell		ssh	
	Hard shell		hsh	
Fragility	Fragile		fra	Proxy for palatability (production)
	Intermediate		int	
	Robust		rob	
Longevity	Very short	<1 year	vsho	Energy fixation, turnover, production rate
	Short	1–2 year	sho	
	Long	2–5 year	lon	
	Very long	>5 year	vlon	
Energy	Low	>1.7 kJ g^{-1}	low	Proxy for quality as food resource
	Medium	1.7–3.4 kJ g^{-1}	med	
	High	3.4-5.1 kJ g^{-1}	high	
Environmental position	Infauna deep	>5 cm	idee	Susceptability to predation, space resource dynamics
	Infauna middle	within 2–5 cm	imid	
	Infauna top	top 2 cm	hop	
	Epibenthic		eben	
	Benthic pelagic		benp	
Feeding position	Suspension		susp	Energy transport: production from pelagos
	Surface		surf	elemental cycling/production bentho-pelagos
	Subsuface		subs	elemental cycling within benthos
	Selective		sel	Decomposition
	Miner		min	
Dispersal habit	Non dispersal	resident	ndis	Production, movement of resources
	Local	10–1000 m	loc	
	Long distance	>1 km	lond	
Sediment transport	No transport		ntra	Habitat modification, bioturbation, elemental cycling within benthos
	Diffusive mixing		difm	
	Surface deposition		surd	
	Conveyer belt transport		cvbt	
	Reverse conveyer belt transport		rcbt	

station as random factor, to account for non-independence of observations from the same stations over time, using the 'nlme' library (Pinheiro et al. 2015) in the R environment. For analysing changes in trait composition and expression, we first calculated the functional identity as community-level weighted means of trait category expressions (CWM). CWM was calculated in the 'FD' library for every sampled station and grouped in exposure-class and year. We tested for differences in CWM between exposure groups and over time: First we used a two-factoral PERMANOVA test design with 'exposure' and 'year' as fixed factors and unrestricted permutations of raw data, including 9999 permutations, to uncover if exposure and time, as well as their interaction, had an effect on the CWM. Next we performed a pair-wise test for pairs of levels of factor 'year' within level of 'exposure' to check for differences in trait expressions within each exposure group over time. To examine if trait expressions differed between exposure levels within years, we did a similar pair-wise test comparing levels of exposure within levels of years. In cases where the functional composition differed significantly we applied a similarity-percentage (SIMPER) -analysis with a 90% accumulative contribution cut-off to highlight which traits were mainly responsible for the observed differences. To test if changes in functional identity followed changes in taxonomic composition, we examined differences in community composition, using the same PERMANOVA designs on square root transformed abundance data. For PERMANOVA- and SIMPER-analyses we used the PRIMER package ver. 6 with PERMANOVA + add on (Clarke and Gorley 2006, Anderson et al. 2008).

Data deposition

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.6hc8q> (Weigel et al. 2016).

Results

Functional dispersion over space and time

We found a total of 27 species at the sheltered and exposed sites. Two species were excluded from the analysis due to their meiobenthic size (passing through a 0.5 mm mesh size) in the northern Baltic, and their rareness in the data (Turbellaria = 0.2% of total species abundance at exposed sites and only present in 1989; *Manayunkia aestuarina* = 0.2% at sheltered sites in 2013; 0.02% at exposed sites in 2013). One species was excluded due to an incomplete set of available trait characteristics (*Planorbis* sp. = 0.04% at sheltered sites in 2000). As the calculated functional measures are based on abundance and are independent of species richness, the exclusion did not affect the general patterns of the analysis.

The species richness within sampled stations varied from 1 to 14 species. Assemblages with relatively few species (≤ 5) showed a broad FDis spectrum, ranging from the minimum to the upper most values (91% of maximum FDis). With increasing numbers of present species per station, FDis seemed to stabilize, following the pattern of a saturation curve (Fig. 1).

The FDis within zoobenthic communities in both sheltered and exposed habitats followed similar trends towards higher and less varying values over time (Fig. 2a). The results of the linear mixed-effect model indicate that exposure class had no effect on FDis ($DF = 16$, $p > 0.1$, $t = -0.9$), while the factor 'year' was significant ($DF = 44$, $p < 0.001$, $t = 3.79$). The development to less variable and higher FDis-values was mainly evident in the later part of the study period (2000–2013). Sheltered areas in particular showed a shift in their variation, whereas exposed areas followed a gradual increase with highest FDis and lowest variation in 2013 (Fig. 2a). Analyzing the same data-set but excluding *Marenzelleria* resulted in higher variability and lower FDis within both communities (Fig. 2b). Following this scenario, neither exposure class ($DF = 16$, $p > 0.1$, $t = -1.0$) nor year ($DF = 44$, $p > 0.1$, $t = 0.8$) had an effect on the communities' FDis.

Changes in functional identity and species composition

We plotted the average CWM of all assemblages to visualize a functional meta-composition for the sheltered and exposed communities over time (Fig. 3, Supplementary material Appendix 3 Fig. A3.1, A3.2).

We found that the factors 'exposure' ($F_1 = 7.12$, $p = 0.0003$) and 'year' ($F_3 = 3.84$, $p = 0.0001$) had significant

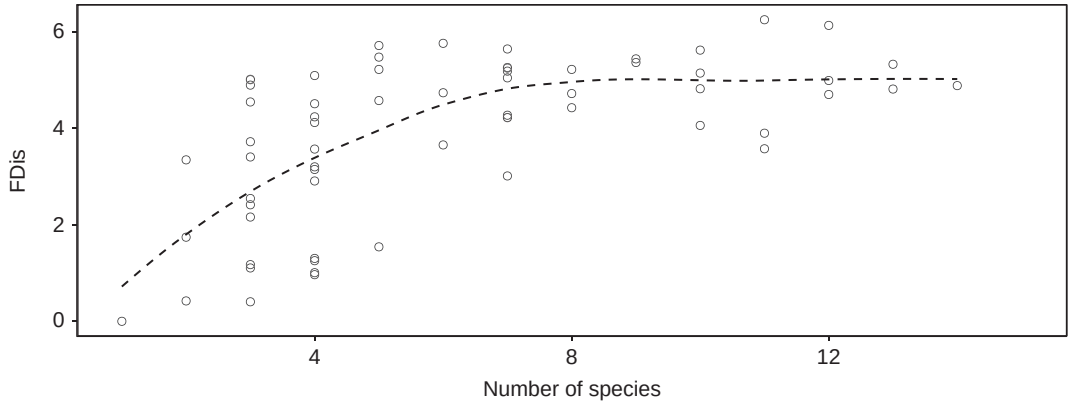

Figure 1. Relationship of functional dispersion (FDis) against the number of species per assemblage for all sampled years (1973, 1989, 2000, 2013). The dashed line represents a fitted local polynomial regression to highlight the relationship.

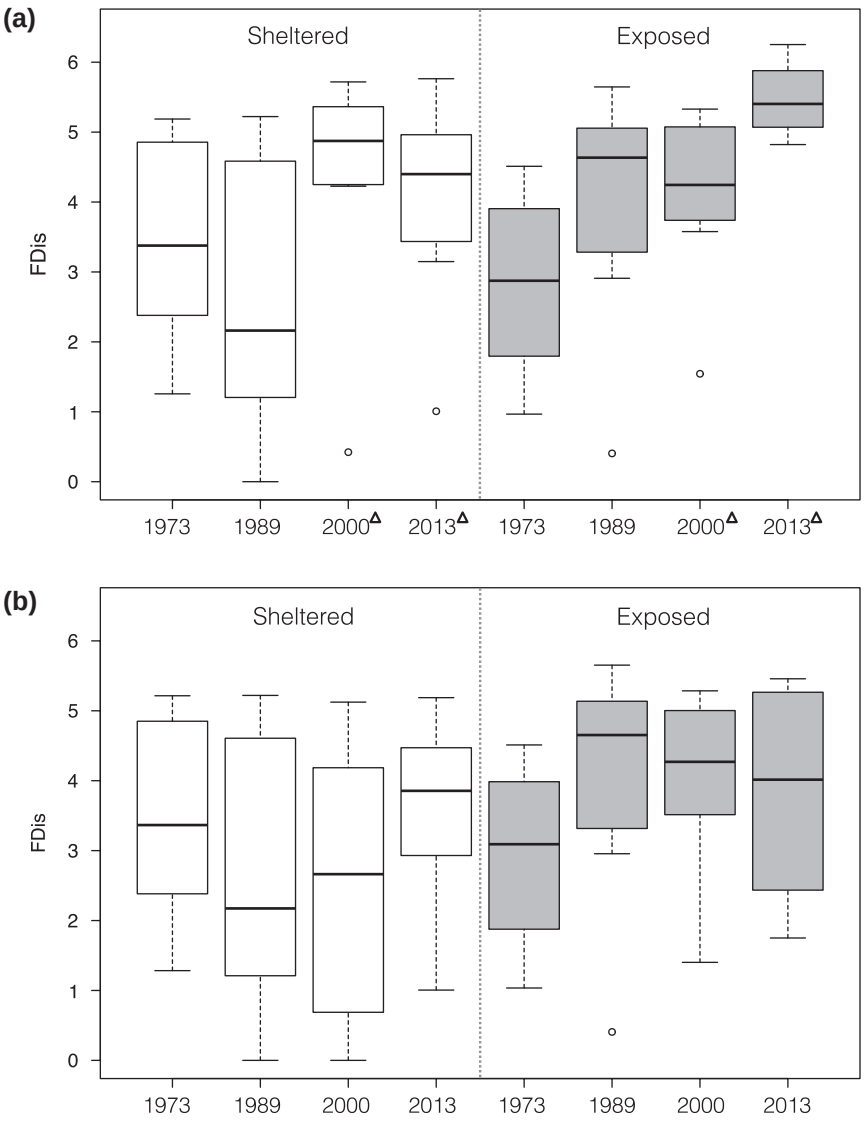

Figure 2. Box-whisker plots of functional dispersion (FDis) in sampled years grouped in exposure classes; white boxes represent sheltered areas (stations n = 8), grey boxes represent exposed areas (stations n = 8); a) shows FDis of full communities complex, b) shows FDis of communities excluding *Marenzelleria* spp, triangles included in years of 2a mark the presence of *Marenzelleria.*

effects on the CWM. Furthermore, their significant interaction ($F_3 = 3.39$, p = 0.0004) indicated that differences within exposure classes were not constant over time. The pair-wise PERMANOVA showed there were no significant changes in functional compositions (functional identity) during the early years (1973 versus 1989) but significant changes over all progressing year-comparisons (1989 versus 2000 and 2000 versus 2013) within the sheltered and exposed communities (Table 2, Fig. 4). Although both systems showed continuous changes over time, from 1989 onward, it is noteworthy that the functional composition within the sheltered stations in 2013 did not differ significantly from the two early years (1973, 1989) in the sheltered area (Table 2). The pair-wise test for differences between sheltered and exposed areas showed that the CWM did not differ significantly across exposure classes in 1973 and 1989, suggesting functionally similar communities in both habitats (Table 3). However, in 2000 and 2013 the differences between functional identities of sheltered and exposed areas became highly significant, pointing towards differently functioning communities in respective areas (Fig. 4). The PERMANOVA on species composition revealed that exposure class ($F_1 = 10.59$, p = 0.0001) and year ($F_3 = 4.95$, p = 0.0001) as well as their interaction ($F_3 = 2.82$, p = 0.0004) had significant effects on the species composition. Contrasting to the results of continuously progressing changes in functional identities from 1989 onward, the communities of sheltered areas showed no significant change in composition between the most recent years (2000 versus 2013). The only significant change in species composition occurred between 1989 and 2000 (Table 3). In exposed areas on the other hand, significant changes in species composition coincided with the shift in functional identity (Table 3). Community compositions between the exposure classes differed from each other in every studied year despite the non-significant differences in their functional identity in the early years of 1973 and 1989 (Table 3).

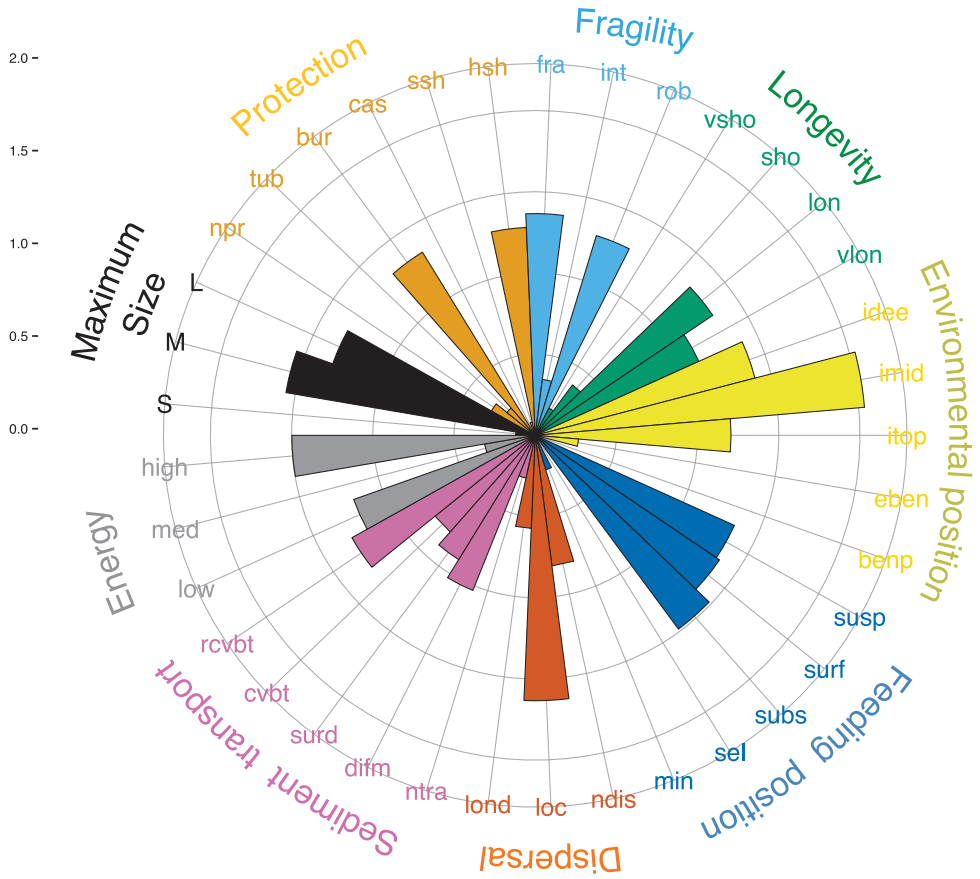

Figure 3. Community-weighted means of trait-category expression (CWM), for all exposed stations in 2013. Colour codes represent the trait affiliation; individual bars represent the trait category expression (for labels see Table 1). (For detailed illustrations of remaining years and exposure classes see Supplementary material Appendix 3 Fig. A3.1, A3.2.).

Examining the significant changes in CWM using SIMPER, we were able to analyse to which extent specific traits and their categories contribute to the observed difference (Table 4). The total percentage of category-contributions within each trait as well as high percentage single categories emphasise the most important traits regarding the change over time.

In the sheltered habitats, the three traits mainly contributing to the change between 1989 and 2000 were 'feeding position', 'protection' and 'fragility'. Within 'feeding position', the expression of suspension and surface feeders as well as miners went down while subsurface feeders increased. The 'protection' trait showed highest decrease in the hard-shell category, while the only increasing category was borrow-protected. The community also became more fragile and less robust. Additionally, we observed a trend towards increasing size of individuals with a higher proportion of large-sized expression. An increase in high 'energy' content was observed while medium and low 'energy' proportions declined. Between the years 2000 and 2013, 'protection', 'feeding position', and 'longevity' were the traits explaining the largest contribution to the difference. We observed a contrasting pattern to the change of previous years in the 'protection' and 'feeding position' traits, where the changing categories showed a reversed trend, back towards their initial state in 1989. Despite that, hard-shelled, surface feeders kept decreasing. Changes in the communities' 'longevity' showed higher proportions in very short- to short-lived organisms

Table 2. Two-factor pairwise PERMANOVA results of year groups within level of exposure (sheltered and exposed) for community weighted means of trait expression (CWM) and species composition. Bold values highlight statistical significance.

	CWM				Species composition			
	Sheltered		Exposed		Sheltered		Exposed	
Groups	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
1973–1989	0.6499	0.6419	1.0427	0.3434	0.5904	0.927	1.1925	0.2241
1973–2000	1.8354	**0.0208**	1.7523	**0.0363**	2.0007	**0.0029**	2.2007	**0.0002**
1973–2013	0.9209	0.4274	3.1637	**0.0014**	1.4848	0.0691	3.3169	**0.0002**
1989–2000	1.8928	**0.0147**	1.8592	**0.0207**	1.9472	**0.003**	1.9944	**0.0005**
1989–2013	1.4879	0.1141	4.1473	**0.0003**	1.6724	**0.0215**	3.2996	**0.0002**
2000–2013	1.6662	**0.0394**	3.0376	**0.0004**	1.3822	0.1095	2.5163	**0.0004**

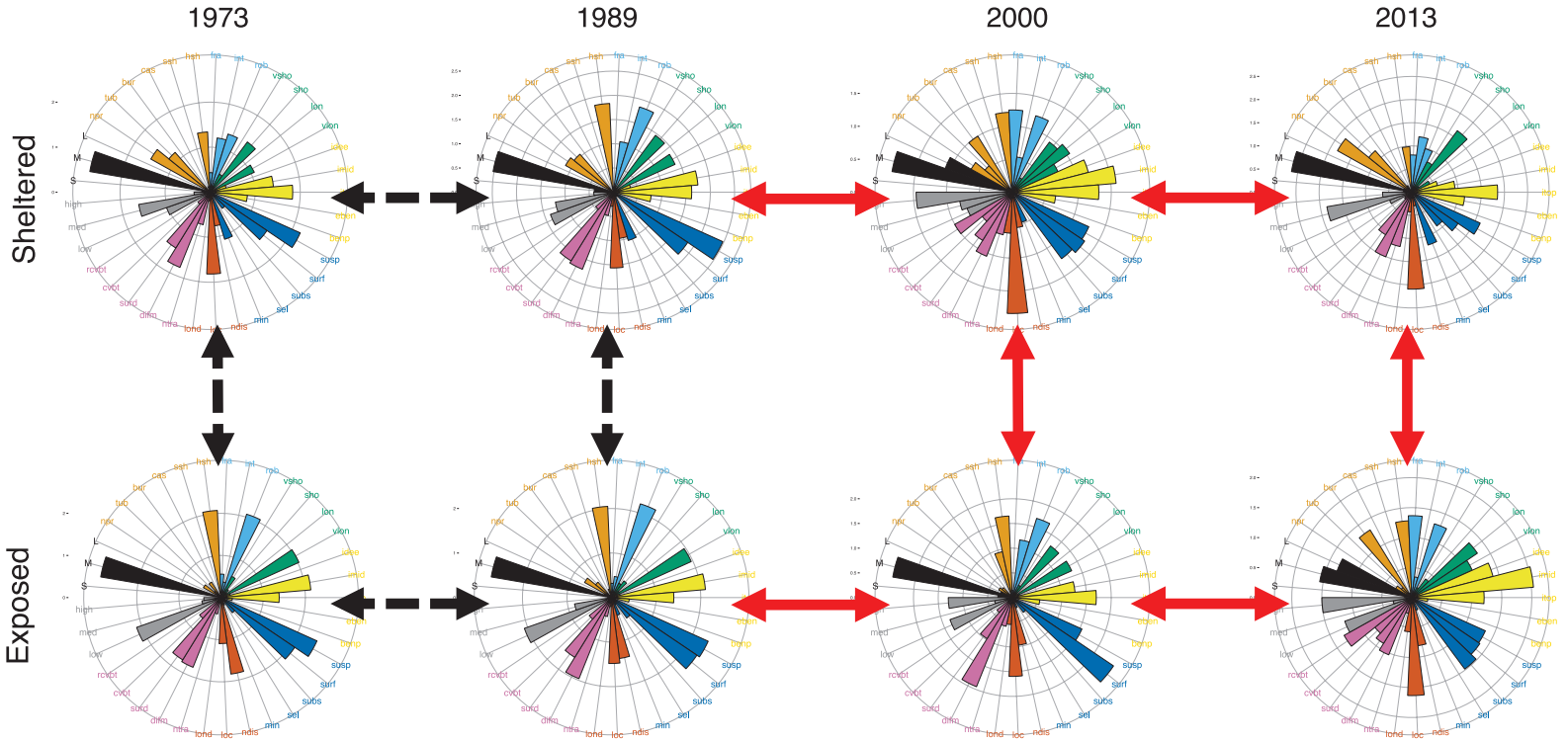

Figure 4. Conceptual illustration of changes in functional identity, measured as community-weighted means of trait expression (CWM), over time and exposure groups; black arrows indicate no significant changes, red arrows indicate significant changes.

Table 3. Two-factor pairwise PERMANOVA results of exposure groups within level of year (1973, 1989, 2000, 2013) for CWM and species composition. Bold values highlight statistical significance.

Groups	1973		1989		2000		2013	
Sheltered-Exposed	t	P(perm)	t	P(perm)	t	P(perm)	t	P(perm)
CWM	1.67	0.0682	1.27	0.2264	1.95	**0.007**	3.17	**0.0013**
Species composition	1.90	**0.0208**	1.56	**0.0136**	2.55	**0.0003**	2.66	**0.0014**

and lower proportions in long- to very long-lived organisms. Energy categories indicated increasing medium and decreasing high and low proportions. The size categories increased in medium-sized organisms while large-sized decreased again (Table 4 Sheltered, Supplementary material Appendix 3 Fig. A3.1).

In the exposed areas the three main traits responsible for the differences between 1989 and 2000 were 'energy' content, 'protection' and 'feeding position'. The strongest contribution to the functionally dissimilarity was the increase in the high-energy and the decrease in the low-energy categories (Table 4 Exposed). Protection patterns changed towards more soft-shelled organisms and less hard-shelled ones. The largest change in the feeding position was due to the decrease in suspension feeders. Within the 'environmental position' trait, species in the upper sediment gained more weight. Size structures remained similar, whereas longevity patterns changed due to an increase in short-lived organisms and a decrease in very long-lived ones. The subsequent temporal change of exposed communities in 2000 and 2013 followed similar trends of the previous development, intensifying the observed trait expressions. The three highest contributors to the change were 'sediment transport', 'environmental position' and 'feeding position'. The altered 'sediment transport' trait showed a signal towards more sediment restructuring as both conveyer-belt-transporters increased, while categories of no transport, diffusive mixing and surface deposition decreased. Contrasting to the change from 1989 to 2000, the main 'environmental position' of the community changed towards higher expressions of middle- to deep-infauna whereas the top-infauna and epibenthic organisms decreased in their community weighing. The change in 'feeding position' was characterised by increasing sub-surface feeders and decreasing suspension and surface feeders. The energy-trait pattern continued towards increased high values. Size structure gained more large-sized organisms while medium-sized ones declined. Additionally, the community-levels of long-lived species increased (Table 4 Exposed, Supplementary material Appendix 3 Fig. A3.2).

Discussion

We show that two zoobenthic communities with diverging development (species abundance, biomass, diversity) maintained equal functional diversities in terms of FDis, pointing toward functionally complex communities in both areas. The invasion of *Marenzelleria* may have promoted a generally higher FDis in both systems. In contrast to the maintained functional diversity, the functional identity of the communities differed significantly over space and time, highlighting shifts in qualitative services for ecosystem functioning. We show the importance of evaluating the present expression of functional traits, serving as proxy for related functions in a species complex, as these focal changes are not captured by functional diversity metrics (e.g. FDis) or community changes.

Functional dispersion of changing communities

Ecosystems with high FDis are assumed to display broader ecosystem functioning (Clark et al. 2012). The FDis of sampled assemblages in the present study suggests two patterns, first, a positive richness–FDis correlation displaying high variation in FDis, until a species complex of roughly five taxa; and second a linear relation with less variation in FDis, independent of species richness (Fig. 1). Less variation in FDis illustrates a higher functional redundancy, as higher species richness was not associated with higher FDis (Cadotte et al. 2011). The high variation in FDis at stations including only three to four taxa suggests a potentially high variation in ecosystem functioning and adaptability to change. This underlines the importance of particular functional compositions, especially in low diverse systems where local assemblages often consist of only a hand full of species.

The community properties of the sheltered and exposed areas investigated in this study have been shown to develop differentially over the past decades, with results traditionally suggesting respectively lower and higher ecological quality status (Weigel et al. 2015). The diversity of expressed functional traits on the other hand remained similar between both exposure classes (Fig. 2). Both areas showed increasing FDis-values over time and a reduction of the within year variation. High FDis with less variation suggests that both areas functionally adapted to their changing surrounding environment over the past decades, showing higher resilience towards varying environmental conditions, while displaying a broader range of ecosystem processes (Tilman et al. 1997, Hulot et al. 2000, Folke et al. 2004, Cadotte et al. 2011). The increased FDis and reduced variation, coincided with the invasion of *Marenzelleria* in the early 1990s (Norkko et al. 1993). Though invasive species are often considered to have negative impacts on native communities, it is important to assess their potential functions and ecological roles to evaluate their possible impact (Kauppi et al. 2015). By omitting *Marenzelleria* from the analysis, the higher and less variable FDis pattern disappeared (Fig. 2a–b). Its presence strongly supported the effect of 'year' on the FDis progression by, reducing the variation while increasing absolute FDis values. It is important to note that *Marenzelleria* did not contribute to a higher FDis by adding new functions, but by strengthening an altered composition of expressed functional traits, in combination with its high abundance. The higher FDis values including *Marenzelleria* are an example of increased niche differentiation (Dimitriadis et al. 2012) and resource

Table 4. SIMPER analysis results of significant changes between year groups within exposure classes with 90% cumulative contribution cut-off. *Av. CWM* indicates the average community weighted mean of trait expression-value of all stations within given year, arrows imply direction of change, *Contrib%* indicates the percentage of contribution to the dissimilarity between years, *total trait contrib.%* is the sum of trait-category contributions within each trait.

		Sheltered										Exposed									
		Average dissimilarity = 48.11					Average dissimilarity = 45.56					Average dissimilarity = 31.10					Average dissimilarity = 41.24				
Trait	Category	1989 Av.CWM	2000 Av.CWM	Trend	Contrib %	Total trait contrib. %	2000 Av.CWM	2013 Av.CWM	Trend	Contrib %	Total trait contrib. %	1989 Av.CWM	2000 Av.CWM	Trend	Contrib %	Total contrib. %	2000 Av.CWM	2013 Av.CWM	Trend	Contrib %	Total trait contrib. %
Maximum size	S	–	–		–	**6.41**	–	–		–	**6.26**	–	–		–	**2.4**	–	–		–	**8.47**
	M	2.52	1.85	↓	3.21		1.85	2.64	↑	2.98		2.74	2.45	↓	2.4		2.45	1.56	↓	4.15	
	L	0.08	1.08	↑	3.2		1.08	0.19	↓	3.28		–	–		–		0.25	1.32	↑	4.32	
Protection	No protection	1.13	0.72	↓	3.56	**14.29**	0.72	1.84	↑	4.95	**15.71**	0.7	0.32	↓	2.8	**13.87**	–	–		–	**11.72**
	Tube	1.05	0.54	↓	3.33		0.54	1.19	↑	3.53		0.46	0.27	↓	2.3		–	–		–	
	Burrow	0	1	↑	3.2		1	0.17	↓	3.16		–	–		–		0.07	1.32	↑	4.87	
	Case	–	–		–		–	–		–		–	–		–		–	–		–	
	Soft shell	–	–		–		–	–		–		0.2	0.95	↑	4.37		0.95	0.08	↓	3.57	
	Hard shell	1.83	1.21	↓	4.2		1.21	0.99	↓	4.07		2.05	1.65	↓	4.4		1.65	1.28	↓	3.28	
Fragility	Fragile	0.11	1.25	↑	3.74	**11.28**	1.25	0.81	↓	3.66	**11.27**		–		–	**9.53**	0.15	1.37	↑	4.76	**11.84**
	Intermediate	1.05	0.54	↓	3.33		0.54	1.21	↑	3.54		0.48	1.18	↑	4.94		1.18	0.35	↓	3.77	
	Robust	1.84	1.21	↓	4.21		1.21	0.99	↓	4.07		2.2	1.68	↓	4.59		1.68	1.29	↓	3.31	
Longevity	Very short	–	–		–	**10.96**	0.25	0.74	↑	2.71	**12.4**	0.36	0.28	↓	1.92	**11.72**	–	–		–	**11.6**
	Short	1.46	0.95	↓	3.83		0.95	1.65	↑	3.71		0.47	1.3	↑	5.27		1.3	0.39	↓	4.05	
	Long	0.05	1.05	↑	3.2		1.05	0.18	↓	3.21			–		–		0.1	1.32	↑	4.73	
	Very long	1.4	0.76	↓	3.93		0.76	0.43	↓	2.77		1.96	1.32	↓	4.53		1.32	1.11	↓	2.82	
Energy	Low	1.38	0.73	↓	3.86	**10.89**	0.73	0.51	↓	2.68	**10.84**	2.08	1.34	↓	5.06	**14.35**	1.34	1.19	↓	2.82	**5.89**
	Medium	1.21	0.81	↓	3.34		0.81	1.86	↑	4.81		0.84	0.36	↓	3.05		–	–		–	
	High	0.41	1.46	↑	3.69		1.46	0.63	↓	3.35		0.08	1.3	↑	6.24		1.3	1.5	↑	3.07	
Environmental position	Infauna deep	0.36	1.19	↑	3.12	**8.17**	1.19	0.58	↓	3.02	**11.23**	–	–		–	**10.54**	0.15	1.4	↑	4.86	**12.78**
	Infauna middle	1.75	1.58	↓	2.75		1.58	0.94	↓	2.93		2.11	1.27	↓	5.08		1.27	2.04	↑	3.49	
	Infauna top	1.62	1.31	↓	2.3		1.31	1.85	↑	3.1		1.4	1.7	↑	3.3		1.7	1.21	↓	2.79	
	Epibenthic	–	–		–		0.66	1.14	↑	2.18		0.4	0.56	↑	2.16		0.56	0.27	↓	1.64	
	Benthic pelagic	–	–		–		–	–		–		–	–		–		–	–		–	
Feeding position	Suspension	2.48	1.27	↓	4.14	**15.4**	1.27	1.62	↑	3.01	**14.14**	2.38	1.54	↓	4.91	**12.04**	1.54	1.35	↓	3.15	**11.94**
	Surface	1.85	1.32	↓	4.11		1.32	1.21	↓	3.92		2.31	2.47	↑	2.76		2.47	1.37	↓	4.38	
	Subsuface	0.12	1.26	↑	3.75		1.26	0.91	↓	3.58		0.57	0.37	↓	2.17		0.37	1.48	↑	4.41	
	Selective	–	–		–							–	–		–		–	–		–	
	Miner	1.04	0.48	↓	3.4		0.48	1.19	↑	3.63		0.42	0.22	↓	2.2		–	–		–	
Dispersal habit	Non dispersal	0.96	0.54	↓	2.68	**2.68**	–	–		–	**0**	1.38	0.97	↓	3.12	**6.81**	0.97	0.81	↓	2.05	**2.05**
	Local	–	–		–		–	–		–		1.48	1.6	↑	1.82		–	–		–	
	Long distance	–	–		–		–	–		–		0.2	0.55	↑	1.87		–	–		–	

(*Continued*)

Table 4. (Continued)

		Sheltered										Exposed									
		Average dissimilarity = 48.11					Average dissimilarity = 45.56					Average dissimilarity = 31.10					Average dissimilarity = 41.24				
Trait	Category	1989 Av.CWM	2000 Av.CWM	Trend	Contrib %	Total trait contrib. %	2000 Av.CWM	2013 Av.CWM	Trend	Contrib %	Total trait contrib. %	1989 Av.CWM	2000 Av.CWM	Trend	Contrib %	Total contrib. %	2000 Av.CWM	2013 Av.CWM	Trend	Contrib %	Total trait contrib. %
Sediment transport	No transport	0.49	0.66	↑	2.3	**10.69**	0.66	1.2	↑	3.34	**8.51**	0.44	0.6	↑	2.95	**8.96**	0.6	0.27	↓	1.88	**14.27**
	Diffusive mixing	1.71	1.05	↓	2.45		–			–		1.96	1.95	↓	2.55		1.95	1.03	↓	3.99	
	Surface deposition	1.67	0.86	↓	2.75		0.86	1.08	↑	2.01		1.58	0.98	↓	3.46		0.98	0.9	↓	1.97	
	Conveyer belt transport	–	–		–		–	–		–		–	–		–		0.4	0.79	↑	1.61	
	Reverse conveyer belt transport	0.01	1	↑	3.19		1	0.17	↓	3.16		–	–		–		0.05	1.29	↑	4.62	

partitioning (Petchey and Gaston 2002, Griffin et al. 2009), with the most abundant species being presumably functionally different and not in direct competition (Mouchet et al. 2010). The approach of excluding *Marenzelleria* to highlight the effect of its absence does however not account for a potential species displacement, and the remaining species compositions could look different without its presence. Yet, there is no evidence that *Marenzelleria* has replaced or directly competed with native species in the northern Baltic (Eriksson Wiklund and Andersson 2014) but rather that it occupied a still open niche following the Baltic Sea post-glacial succession theory (Bonsdorff 2006).

Functional identity of changing communities

Although the diversity of functions between communities with different properties can remain alike, as indicated by similar FDis-levels, their underlying functional identity in terms of qualitative services for ecosystem functioning may be different. Differential strengths of particular trait expressions may alter predator–prey dynamics (Green and Côté 2014), remineralisation of nutrients or organic matter, sediment oxygenation (Karlson et al. 2007, Norling et al. 2007) and system productivity (Roscher et al. 2012).

Our results show that the functional identity within and across the sheltered and exposed communities was statistically indistinguishable in the years 1973 and 1989 (Table 2, 3, Fig. 4), suggesting functionally similar communities in the whole coastal system, despite consistently different species compositions between the two areas (Table 3). This could be explained by findings in Clare et al. (2015) who showed that ecological functioning could be sustained despite compositional changes, possibly due to functionally redundant taxa. However, we also show a contradicting pattern by providing strong evidence for a progressing functional change in the communities, irrespective of compositional changes (Table 2, 3). Even though the system was characterised by high functional redundancy, the functional identities, i.e. the structure of expressed traits, showed high flexibility. We conclude that differences in functional identity are to some extent independent from compositional changes of species, highlighting the need to analyse the expression of traits when investigating changes in the functionality of communities.

Depending on the selection of traits, the expressed functional properties of a species complex can help shed light on its ecosystem value from different perspectives (Dimitriadis et al. 2012). We selected nine functional traits related to different ecosystem services (Table 1) to gain insight into the potentially altered quality of zoobenthos as food-resource for fish, as well as into its properties related to production and role as bioengineer. Size is considered as one of the most fundamental traits as it influences the magnitude of many other traits and their related processes (Eklöf et al. 2013, Norkko et al. 2013). Especially from a predatory perspective prey-size plays a key role (Klecka and Boukal 2013, Green and Côté 2014, Nordström et al. 2015). Smaller prey taxa have generally a higher number of predators feeding on them (Nordström et al. 2015) and are therefore under higher predation pressure. The range of protection, fragility, and environmental position give indication for prey accessibility and

the ease of its ingestion. Following the optimal foraging theory, predators are likely to select prey that provide them with highest energetic gain while losing minimal energy along the catching and handling process (Pyke et al. 1977). Consequently, prey species with a relatively high energy content should be preferred in cases where their abundances are equal to lower energy species.

In 2013, the sheltered sites where primarily characterized by one size class (medium), short lived species with no protection, being less robust and occurring in the top 2 cm of the sediment or epibenthically. Although the energy trait varied, we detected a continuous decrease in low energy taxa leading to increased energy contents, which in addition to the previously mentioned development points towards easier accessible (size, position in sediment) and digestible (no protection) prey items, with higher turnover rates (short-lived).

The most pronounced changes in the exposed sites were due to continuously increasing high-energy values and higher fragility, which despite the fauna being positioned deeper in the sediment still suggest higher food quality, especially when biomass is high as well.

The feeding position gives indication for productivity pathways and the strength of benthic–pelagic coupling (Dimitriadis et al. 2012). It was the only trait, always being among the most contributing ones for changes in functions between the year groups in both systems (Table 4), indicating that the systems productivity is governed by different pathways in relation to environmental change (Tomczak et al. 2012). In the 1970s and 1980s, both systems were primarily represented by suspension and surface feeders suggesting a strong dependency on the productivity within the water column and benthic–pelagic coupling (Kristensen et al. 2014). During the past decades, the relative importance of those feeding categories decreased, which could be linked to gradually decreasing levels of eutrophication compared to the 1970s (HELCOM 2013). With increased importance of sub-surface feeders in both systems, and a more even expression of various feeding types, we show that the main energy pathways changed towards being diverse and less restricted (Pacheco et al. 2011). The increase in sub-surface feeders, especially in the exposed areas, suggests a higher extent of sediment reworking and ventilation (Kristensen et al. 2012), which is linked to recycling of nutrients and organic matter (Meysman et al. 2006). The sediment transport characteristics reinforced increased bioturbation rates by showing strong increases in conveyor-transporters in exposed sites. Sheltered sites on the other hand have functionally returned to their initial state of the 1970s and 1980s, being dominated by diffusive mixers with increased expressions of the non-sediment transport category, implying less reworking of sediments (Table 4, Supplementary material Appendix 3 Fig. A3.1, A3.2).

Applying the complimenting functional measures used in this study (FDis and CWM), along traditional taxonomic community changes, increases the understanding of temporal developments of communities in dynamic ecosystems and can help reveal alterations in qualitative services for ecosystem functioning.

Conclusion

Our work highlights the functional identity (composition of expressed traits as CWM) of assemblages and communities as a proxy for ecosystem performance. Using functional diversity metrics alone as ecosystem functioning measures provides only restricted information. We show that two systems, affected differentially by environmental drivers, with different succession in taxonomic composition, biomass and species richness, can still show similar patterns of high functional diversity. While diversity measures do not provide information on the underlying functional identity, we also exemplify that equally functionally diverse ecosystems may develop in diverging directions with different underlying functional expressions. Hence, our approach provides a quantitative measure to the qualitative functions of interest.

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Supplementary material (available online as Appendix oik-02894 at <www.oikosjournal.org/appendix/oik-02894>). Appendix 1. Figure A1: Map of the Åland Islands including all sampling sites. Appendix 2. Table A1: Species energy value approximations and categories for the 'energy' trait. Appendix 3. Figure A3.1 and A3.2: Detailed illustrations of community weighted means of trait expression (CWM) in sheltered and exposed habitats for all sampled years.

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Appendix 7.

Hydrobiologia
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PRIMARY RESEARCH PAPER

Feeding patterns of dominating small pelagic fish in the Gulf of Riga, Baltic Sea

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Abstract We investigated the feeding of the dominant small pelagic fish—herring *Clupea harengus membras* and three-spined stickleback *Gasterosteus aculeatus*—in the Gulf of Riga (Baltic Sea) in the summers of 1999–2014. The share of empty stomachs, stomach fullness and taxonomic composition of fish diet was analysed. On average, large herring had the highest (19%) and small herring the lowest (6%) share of empty stomachs. Small (<1 mm) cladoceran *Bosmina* spp. was the most important prey for three-spined stickleback; preying on small (<1.5 mm) copepod *Eurytemora affinis* was the most efficient for small herring, while *Bosmina* spp. and *E. affinis* were equally important for the large herring, followed by the large (mean body length <2.0 mm) non-indigenous cladoceran *Cercopagis pengoi*. The number of prey taxa per stomach exhibited significant differences between the fish groups studied; the highest mean value was recorded for small herring and the lowest for three-spined stickleback (2.1 and 1.4 taxa, respectively). Although present, the fish group-specific spatial dynamics in feeding parameters (share of empty stomachs and feeding intensity) were weak compared to the observed interannual variation.

Keywords Herring *Clupea harengus membras* · Three-spined stickleback *Gasterosteus aculeatus* · Diet composition · Feeding activity · Diversity of stomach content

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Introduction

Process-based understanding of the dynamic interactions within the food web and pathways from lower to higher trophic levels precede successful ecosystem-based management. Small pelagic fish are important elements in these processes in marine ecosystems. They can significantly structure the ecosystems via selective feeding on a range of planktonic organisms and transfer energy to larger, piscivorous species (van der Lingen et al., 2009). Prey availability and density affect the survival and reproduction of pelagic fish at individual and population levels, which can lead to changes in the food web (Rönkkönen et al., 2004). From the top-down perspective, small pelagic fish may affect both zooplankton production (Rudstam et al., 1994) and densities of early life stages of ecologically

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and commercially important fish populations (Köster et al., 2005; Byström et al., 2015). These processes and their ecological relevance are dynamic at various spatial and temporal scales, and may be modulated by changing environmental conditions (Stige et al., 2014).

Two general approaches are usually applied to understand the predator–prey relationships of fish. The first is the mechanistic approach that uses functional analysis to determine how the fish detects and consumes their prey. The second is a theoretical approach, predicting prey choice on the basis of gain and loss of energy and time. These two approaches identify the set of conditions under which the predator will maximize its ecological fitness (Hughes, 1997; Wootton, 1998), and predict how the predator should choose its prey most efficiently.

The Baltic Sea is a species-poor brackish system with only a few abundant pelagic fish species present. Throughout the last two decades, the clupeids herring *Clupea harengus membras* Linnaeus, 1761, and sprat *Sprattus sprattus* (Linnaeus, 1758) dominate in the open sea, with three-spined stickleback *Gasterosteus aculeatus* (Linnaeus, 1758) being more abundant in shallow coastal areas. Several aspects of feeding ecology of clupeids are well studied, due to important implications for potential density dependency and associated individual- and population-level performance (e.g. Arrhenius, 1996; Cardinale & Arrhenius, 2000; Kornilovs et al., 2001; Casini et al., 2004; Möllmann et al., 2004). In contrast, notably less is known about the feeding of non-commercial three-spined stickleback: they may also occur in relatively high quantities at certain times and areas, and their diet may substantially overlap with clupeids (Kostrichkina, 1970; Ojaveer et al., 1997; Peltonen et al., 2004), suggesting competition for food at individual and population level when prey is scarce. Herring and three-spined stickleback are both visual feeders—they detect and select their prey based upon visual factors related to abiotic (e.g. visibility and light regime) as well as biotic environment, including prey density, size, colour, shape, palatability and motion (Zaret & Kerfoot, 1980; Aksnes & Giske, 1993; Strickler et al., 2005).

The aim of the current study was to investigate the individual and spatial patterns of feeding of the two strongly dominating zooplanktivores—herring and three-spined stickleback—on a basin-wide scale in the Gulf of Riga, Baltic Sea, based on the stomach content analyses. Considering the similar trophic position of the two fish species, we expected that (i) pelagic fish mostly consume a few and overlapping prey items in this species-poor environment, (ii) small-sized brackish warm water crustaceans that dominate in zooplankton samples also dominate in fish diets, and (iii) trophic interactions involving pelagic fish may exhibit spatial and temporal patterns, which should advise the assessment and management of the GoR ecosystem.

Materials and methods

Study area

The Gulf of Riga (GoR, Fig. 1) is a semi-enclosed shallow basin (mean 26, maximum >60 m, area 17,913 km^2, and volume 405 km^3) in the northeastern Baltic Sea (Leppäranta & Myrberg, 2009). It is connected to the Baltic Proper via two shallow straits in the north-west, and receives most of the freshwater from rivers in the south, 70% of all riverine inflow originates from Daugava River (Yurkovskis et al., 1993). Mean salinity is low (5.6 psu), and the estimated water renewal period is three years (Lilover et al., 1998). The gulf is ice-covered in winter. The

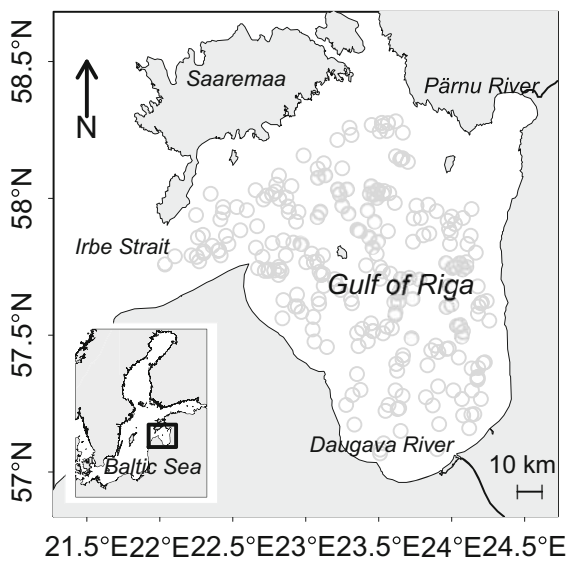

Fig. 1 Map of the study area with location of trawls in the Gulf of Riga, in summer 1999–2014

seasonal thermocline occurs from May until September and separates the relatively stable deep water (temperature ca. 3°C) and the highly variable surface layers (temperature up to 20°C) (Raudsepp, 2001). The GoR is one of the most eutrophicated regions in the Baltic Sea (HELCOM, 2010).

Sampling and sample analysis

Fish were collected during hydroacoustic surveys that estimated the size of commercially important pelagic fish stocks in the GoR from 1999 to 2014. Surveys always took place in the second half of July. In total, 264 trawl hauls were conducted (Table 1; Fig. 1, Supplementary Material 1) using a pelagic commercial trawl (midwater (stern) otter trawl (OTM_SPF), mesh size 10 mm) in 30-min hauls at ca. 5.6 km h^{-1} speed. Sampling was targeted at locations where the highest biomasses of fish were observed. Specimens were measured for total length to the nearest mm, and individual body mass was determined with the precision of 0.1 g. Stomachs of up to 20 randomly chosen individuals per haul from each target group—small herring (immature juvenile fish, 4–9 cm), large herring (immature fish at the stage of early development and maturing fish at the stage of early vitellogenesis/spermatogenesis, 10–17 cm) (ICES, 2011) and three-spined stickleback (4–8 cm)—were stored in a 4% formaldehyde solution until the analysis.

Stomach content analysis was performed according to the method proposed by Melnitchuk (1980), which requires investigation of the whole stomach content for taxonomic composition and determination of biomass of each taxa found in stomachs. The analysis was performed under a light microscope (50–100 × magnification). The volume share of the small-sized prey (i.e. copepods and small cladocerans) was visually estimated, and this was then used as a proxy for the actual contribution to the stomach content biomass. The total stomach content and larger prey types, e.g. mysids, amphipods, *Cercopagis pengoi* (Ostroumov, 1891) and fish larvae, were weighed to a precision of 0.001 g. All prey items were determined to the lowest possible taxonomic level. In total, 5,215 large herring, 1,171 small herring and 3,266 three-spined stickleback were used in the analysis (Table 1).

Mesozooplankton was sampled subsequently to trawling during 2001–2013. The samples were collected by vertical hauls covering the whole water column with a large Juday net (mouth surface area 0.1 m^2, mesh size 90 µm) and preserved in a 4% formaldehyde solution. Sample analyses were performed according to guidelines outlined by HELCOM (1988). The following five zooplankton taxa, which

Table 1 Number of trawls performed, together with the number of fish used for stomach content analysis

Year	Number of trawls	Small herring	Large herring	Three-spined stickleback
1999	12	0	231	220
2000	12	43	205	218
2001	12	24	227	200
2002	14	273	250	90
2003	18	38	398	300
2004	15	47	333	180
2005	16	130	303	280
2006	17	10	398	260
2007	15	149	296	220
2008	18	11	366	180
2009	19	20	374	78
2010	18	13	360	260
2011	20	273	397	360
2012	19	81	367	100
2013	18	0	347	60
2014	21	59	363	260
Total	264	1171	5215	3266

played the major role in fish diets, were subjected to further analysis: the calanoid copepods *Eurytemora affinis* (Poppe, 1880) and *Acartia* spp., and the cladocerans *Bosmina* spp., *C. pengoi* and *Pleopis/Podon* spp.

Data analysis

The stomach content analyses were performed in three levels in terms of the selection of data: (i) analyses that included the data from all stomachs (share of empty stomachs), (ii) analyses including only the data from non-empty stomachs (stomach fullness and prey composition) and (iii) prey taxon-specific analyses with data from stomachs where the particular prey dominated. Such differential use of data was chosen to reduce the redundancy in the results, e.g. by excluding the effect of empty stomachs from the average stomach fullness or prey frequency patterns when their share has already been indicated separately.

Analyses using data from all stomachs

The share of empty stomachs (non-fed fish) was expressed as percentage of the total number of stomachs examined. Spatial patterns of the share of empty stomachs were visualized with a Generalized Additive Model (GAM), using the tensor product smoother of latitude and longitude (smooth term *te* in the function *gam* in R library *mgcv*) that returns a two-dimensional surface of fitted values. The fitted values were then interpolated over the area covered with trawls, using the function *krige.conv* in the R library *geoR*.

Analyses using data from non-empty stomachs

Feeding intensity was measured as stomach fullness (SF), calculated first for each individual, and averaged per trawl when identifying spatial patterns. Individual stomach fullness was calculated as

$$\mathrm{SF} = 100 M_{\mathrm{S}} M_{\mathrm{F}}^{-1},$$

where M_{S} is the total stomach content wet mass and M_{F} the fish wet mass in grams.

The diet patterns of three fish groups were described by the frequency of occurrence (F) and the relative proportion (I) of each prey item in the stomachs. The frequency of occurrence was calculated as

$$F = 100 n N^{-1},$$

where n is the number of fish with particular zooplankton taxa in their stomachs and N is the total number of fish with non-empty stomachs.

The relative proportion of prey items in the stomachs was calculated as:

$$I = 100 S_{\mathrm{Z}} S_{\mathrm{T}}^{-1},$$

where S_{Z} is the mass of a given zooplankton taxa in the stomach and S_{T} is the total stomach content mass.

We also evaluated the taxonomic diversity of stomach content as a number of zooplankton taxa found in stomachs. Spatial patterns of the mean stomach fullness index per trawl were visualized in the same way (with the tensor product smoother of latitude and longitude) as the spatial distribution of the share of empty stomachs. Ontogenetic shifts in the diet of herring were estimated with univariate GAM smoothers, fitted to the proportion of each prey taxa as function of the fish body length. Large and small herring were pooled, since the size of the specimen was used as a continuous predictor, but only data from years 2002, 2005, 2007 and 2011 were used, when both size groups were comparably represented (Table 1). Since the size distribution of three-spined stickleback is biased towards larger individuals due to sampling gear, and was further represented mostly by 5–6 cm long individuals, size-dependent feeding was not analysed for this species.

The effect of the interannual variation in relative abundance of different prey taxa in the water column on their prevalence in non-empty fish stomachs was tested with simple linear correlations between the annual mean proportions of each taxon in zooplankton samples and fish stomachs. Annual mean proportion of each taxon in stomachs was calculated as arithmetic mean proportion from all non-empty stomachs and represented the mean proportion of the biomass, while the mean proportions in zooplankton samples were based on the abundances.

Prey taxon-specific analyses

Importance of different zooplankton taxa as prey items was estimated with the one-way ANOVA, using the

dominant prey taxon as categorical explanatory variable, and stomach fullness data of those specimens where one zooplankton taxon clearly dominated (constituted >90%) the stomach content.

Results

Diet composition

From the total of 9,652 stomachs, 1,424 were empty, in remaining 8,228 stomachs, 26 different prey items were identified. Most frequent of these were the cladoceran *Bosmina* spp. (present in >50% of all non-empty stomachs), followed by the copepod *E. affinis* (36.7%), the large predatory cladoceran *C. pengoi* (21.0%), the copepod *Acartia* spp. (16.7%) and the small cladoceran *Podon*/*Pleopis* spp. (8.7%). Full list of taxa and their frequencies in the non-empty stomachs of three fish groups are given in Table 2.

Judging from the relative proportion (*I*) and frequency of occurrence (*F*), *Bosmina* spp. and *E. affinis* were the most consumed prey items for all three fish groups (Fig. 2). In the diets of three-spined stickleback and large herring, *C. pengoi* also contributed substantially, while *Acartia* spp. was more important in the small herring (Fig. 2).

Relative proportion of main prey items was also related to body length in herring: the proportion of *Acartia* spp. declined, after peaking at 8 cm, while the proportion of *C. pengoi* consistently increased with increasing body length. Consumption of *Bosmina* spp. and *Podon*/*Pleopis* spp. exhibited unimodal pattern with peaks at around 9 cm, and *E. affinis* displayed bimodal pattern with opposite dynamics compared to that of *Bosmina* spp. (Figure 3). In addition, several large-bodied taxa such as mysids (*Mysis mixta* Liljeborg, 1853, *M. relicta* Lovén, 1862, *Neomysis integer* (Leach, 1814)), copepods (*Limnocalanus macrurus* Sars G.O., 1863) and gammarids (*Pontoporeia femorata* Krøyer, 1842 and *Monoporeia affinis* (Lindström, 1855)) were recorded only in stomachs of large (≥15 cm) herring (data not shown).

Diversity of stomach content

Taxonomic richness, i.e. the number of prey taxa per stomach, was rarely more than three, and on average 2.1, 1.8 and 1.4 prey taxa were found in the stomachs of small and large herring and three-spined stickleback (Fig. 4). When ranking by the frequency the certain combinations of taxa in small herring stomachs, 21% of cases consisted of single prey—*Bosmina* spp., followed by combination of *Bosmina* spp. + *E.*

Table 2 Most frequently encountered prey items in all non-empty stomachs of the three studied fish groups. As more than one prey was frequently found in the stomach, the sum of percentages exceeds 100

Small herring	Large herring	Three-spined stickleback
Bosmina spp. (71%)	*E. affinis* (42%)	*Bosmina* spp. (66%)
E. affinis (47%)	*Bosmina* spp. (38%)	*E. affinis* (23%)
Acartia spp. (47%)	*C. pengoi* (27%)	*C. pengoi* (17%)
Pleopis/Podon spp. (11%)	*Acartia* spp. (18%)	*Pleopis/Podon* spp. (10%)
Mesocyclops sp. (10%)	*Pleopis/Podon* spp. (7%)	*Acartia* spp. (4%)
Cyclops spp. (4%)	*Mesocyclops* sp. (4%)	Ostracoda (2%)
C. pengoi (3%)	*L. macrurus* (3%)	*Mesocyclops* sp., Harpacticoida, *Evadne* spp, Lamellibranchiata, Diptera, *L. macrurus*, *T. longicornis*, *N. integer*, *Cyclops* spp., *C. h. membras, Pomatoschistus* spp. (each <1%)
Evadne spp. (3%)	Ostracoda (1%)	
Ostracoda (1%)	*P. femorata*, *Evadne* spp. Lamellibranchiata, Harpacticoida, *Cyclops* spp., *M. mixta*, *M. relicta*, *T. longicornis*, *N. integer*, *Gammarus* sp. *C. h. membras, G. aculeatus, O. eperlanus, Pomatoschistus* spp. (each <1%)	
Lamellibranchiata (1%)		
Harpacticoida, *N. integer*, *L. macrurus* (each <1%)		

The species not described in detail in results and discussion belong to following higher groups: Copepoda (*Mesocyclops* sp., *Cyclops* spp., *Temora longicornis* (Müller O.F., 1785), Harpacticoida); Ostracoda; Cladocera (*Evadne* spp.); Gammaridea (*Gammarus* sp.), Polychaeta (*Harmothoe sarsi* (Kinberg in Malmgren, 1865); Isopoda (*Idotea balthica* (Pallas, 1772)); Balanidae (*Amphibalanus improvisus* (Darwin, 1854)); Mollusca (Lamellibranchiata) and fish (*C. h. membras*, *G. aculeatus*, *Osmerus eperlanus* (Linnaeus, 1758) and *Pomatoschistus* spp.)

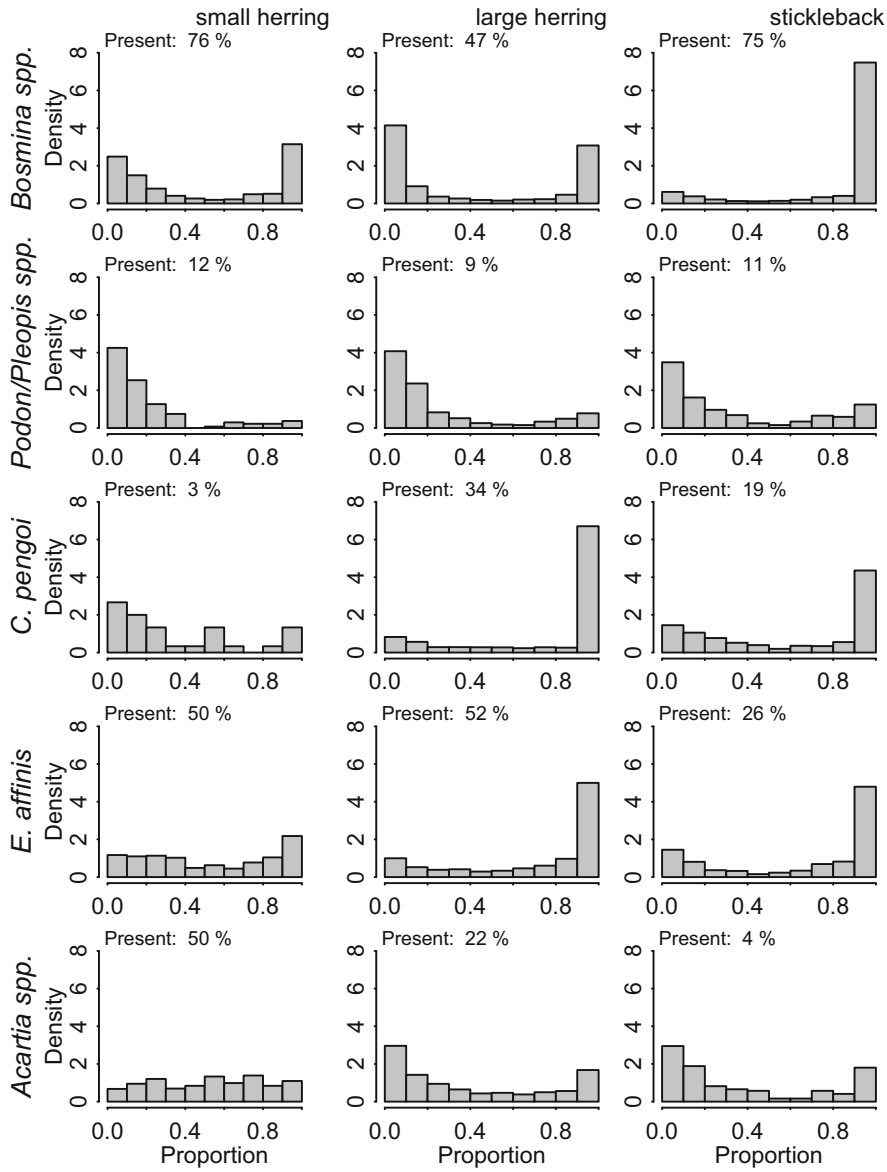

Fig. 2 Distribution of five dominant prey taxa in the non-empty stomachs of the three analysed fish groups (three-spined stickleback *G. aculeatus* and small and large herring *C. h. membras*, see the titles of columns). The % value at the top of each plot denotes the frequency of particular prey item in non-empty stomachs of a given fish group; histograms visualize the typical proportions of each prey item. Histograms are constructed as probability densities for better visual comparison (all have an area one), since different prey taxa were present in different number of stomachs

affinis + *Acartia* spp. (16%), *Bosmina* spp. + *Acartia* spp. (12%), *Bosmina* spp. + *E. affinis* (11%), *Podon*/*Pleopis* spp. (7%) and *Podon*/*Pleopis* spp. + *Acartia* spp. (7%). Thus, small herring was consumed mostly either as *Bosmina* spp. alone, or in different combinations with the two small-sized copepod taxa.

When judging from the stomach fullness, *E. affinis* was the most important prey for both small and large herring, and *Bosmina* spp. for three-spined stickleback. This was evidenced by the fact that consuming mostly the given prey led to significantly higher stomach fullness index than any other prey item (Fig. 5). The exceptions were *Bosmina* spp. and *E. affinis* in the stomachs of large herring that led to equal stomach fullnesses.

Feeding activity

Large herring had the highest (19%) and small herring the lowest (6%) share of empty stomachs. The percentage of empty stomachs differed significantly by fish groups—differences in the percentage of empty stomachs were 13.5, 4.7 and 7.0 ($P < 0.01$), when

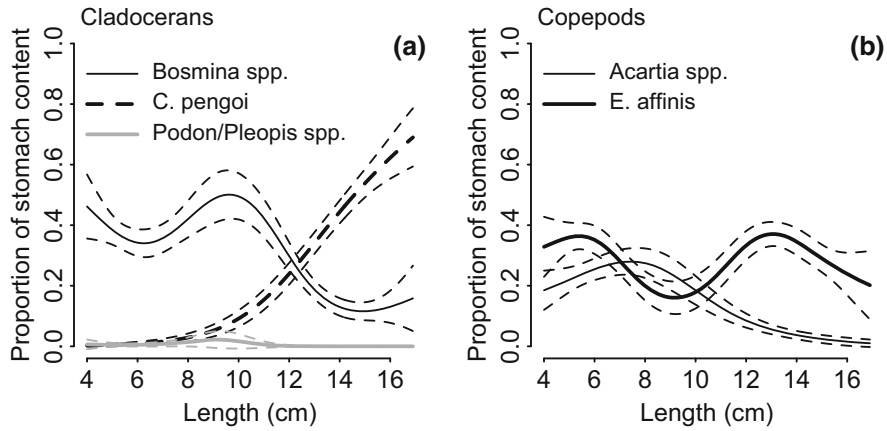

Fig. 3 Size-specific feeding of herring *C. h. membras* on *E. affinis*, *Acartia* spp., *Bosmina* spp., *Podon/Pleopis* spp. and *C. pengoi* in the Gulf of Riga. Due to low representation of small herring in most of the years, only the data from years 2002, 2005, 2007 and 2011 were used here to ensure balanced length distribution of fish. Calculations are based on the non-empty stomachs

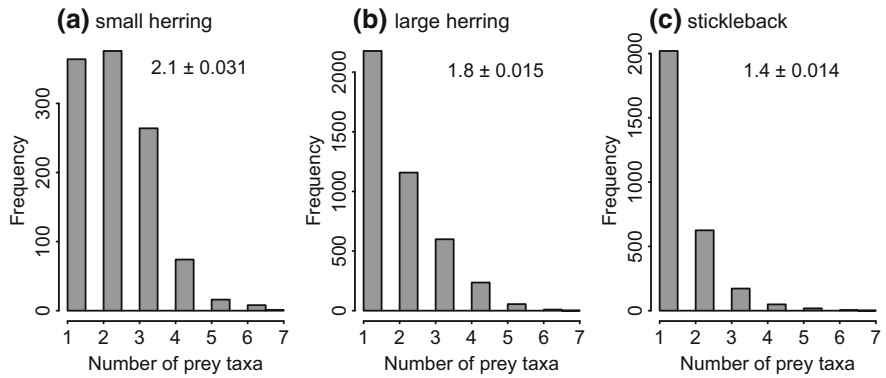

Fig. 4 Taxonomic diversity, measured as the number of zooplankton taxa recorded per non-empty stomach in small (**a**) and large (**b**) herring *C. h. membras*, and three-spined stickleback *G. aculeatus* (**c**)

comparing large herring with small herring, large herring with three-spined stickleback and three-spined stickleback with small herring. The mean stomach fullness index increased with decreasing body size, from large to small herring to sticklebacks (Fig. 6).

Spatial patterns

Feeding conditions for small herring, as revealed by the proportion of empty stomachs and stomach fullness, were relatively uniform across the GoR with the highest stomach fullness observed in the western part of the GoR. Small herring also had the lowest proportion of empty stomachs of the three fish groups studied. In contrast, large herring feeding conditions improved in the northeastern part of the basin where the proportion of empty stomachs is the lowest, while the wider east coast of the basin seems to support higher stomach fullness index. The lowest share of empty stomachs of three-spined stickleback was recorded in the southern part of the basin adjacent to plume of River Daugava, and the best feeding conditions in terms of stomach fullness index are concentrated to the southwestern GoR (Fig. 7). However, the R^2 of the GAMs of spatial patterns was low (0.03–0.11); hence, the spatial patterns are quite weak and interannual variation dominates.

Relation between prey availability and stomach content composition

Elevated abundance of the two most abundant prey taxa—*Bosmina* spp. and *E. affinis*—in the field samples resulted in a consistently higher proportion of these taxa in the stomach content of three-spined stickleback and large herring, but not in small herring. In field samples, *C. pengoi* was always present in low proportion, but was more variable in stomachs of adult herring and three-spined stickleback. This is likely caused by its relatively higher body size compared to other taxa considered here, since the proportion in stomachs represented biomass proportion, while the

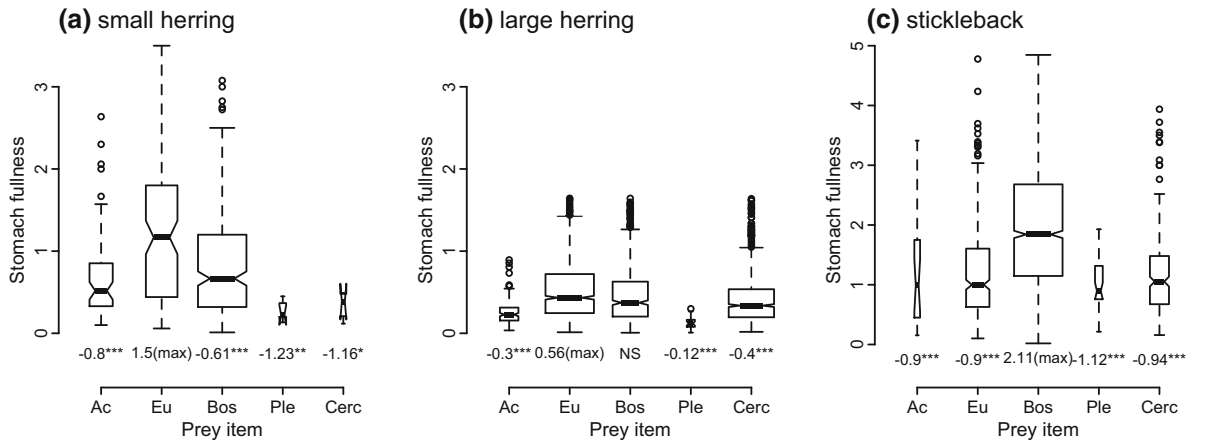

Fig. 5 Importance of different prey taxa for the studied fish groups, based on the fullness of the non-empty stomachs in dominance of single prey (>90% of stomach content): "Ac" *Acartia* spp., "Eu" *E. affinis*, "Bos" *Bosmina* spp., "Ple" *Pleopis/Podon* spp., "Cerc" *C. pengoi.* One-way ANOVA was used to test the significance of the stomach fullness differences, setting the taxon with highest mean stomach fullness (*E. affinis* in small and large herring, and *Bosmina* spp. in three-spined stickleback), as the base level. The numbers under the boxes mark the mean stomach fullness of the prey that yielded highest stomach fullness (marked with "max"), when dominating, and the difference of stomach fullness from that base level for rest of the taxa. Asterisks mark the significance of that difference: '***' = 0, '**' = 0.001, '*' = 0.01. Width of the boxes corresponds to number of stomachs where each prey item dominated. The horizontal bar in the middle of each box is the median; box marks the quartiles (25th and 75th percentiles) of values, the notches extend to ±1.58 interquartile range/sqrt(n) and the whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box

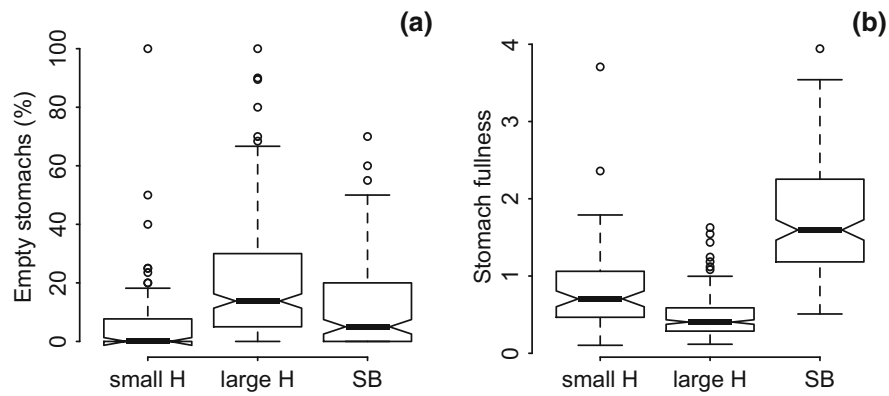

Fig. 6 Feeding intensity of three-spined stickleback *G. aculeatus* (SB), small and large herring *C. h. membras* (small H and large H, respectively) as the percentage of empty stomachs (**a**) and mean fullness of non-empty stomachs (SF) per trawl (**b**). The horizontal bar in the middle of each box is the median; box marks the quartiles (25th and 75th percentiles) of values, the notches extend to ±1.58 interquartile range/sqrt(n) and the whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box

proportion in field samples was calculated from abundance. Share of *Pleopis*/*Podon* spp. was low in the field samples as well as in pelagic fish stomachs, although in the rare years when its proportion was higher in field samples, it was also detected more abundantly in stomachs (Fig. 8).

Discussion

The current study provides new insights to the species-specific and spatial patterns of feeding activity of the dominating pelagic fish (herring and three-spined stickleback) in a distinct sub-basin of the Baltic Sea.

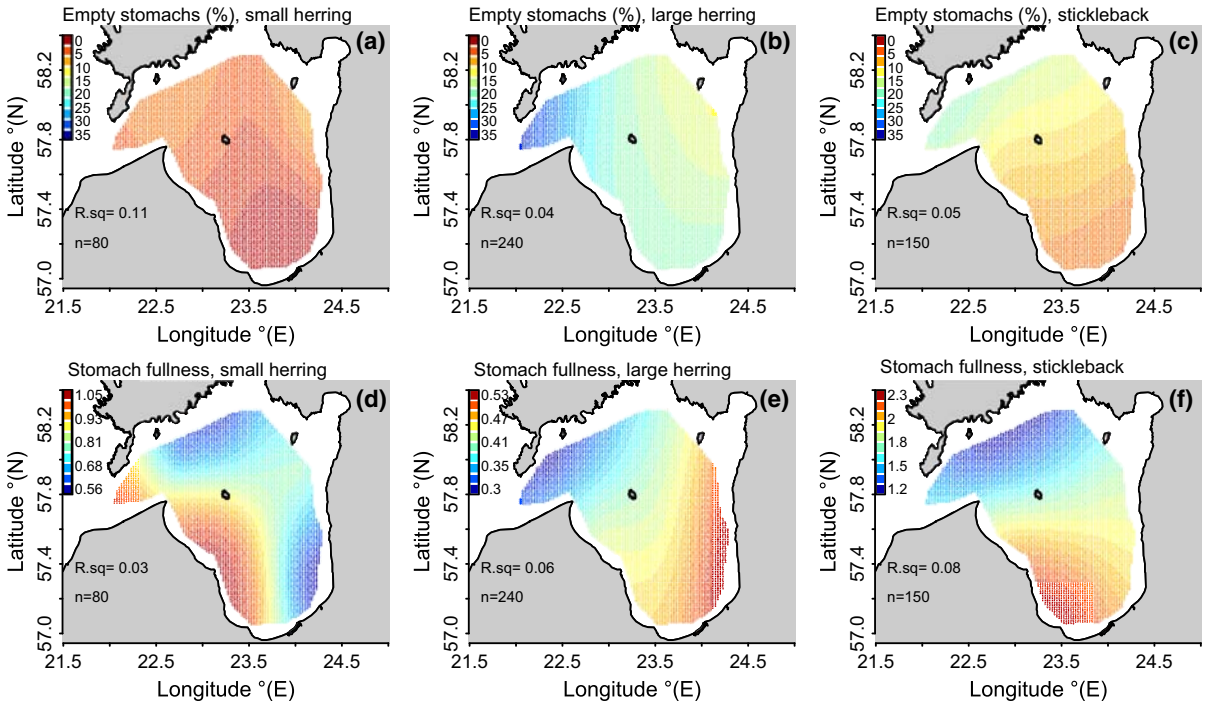

Fig. 7 Spatial patterns of the empty stomachs (**a**–**c**) and mean fullness of the non-empty stomachs (**d**–**f**) of small and large herring *C. h. membras*, and three-spined stickleback *G. aculeatus.* In **a**–**c**, same colour scale has been used

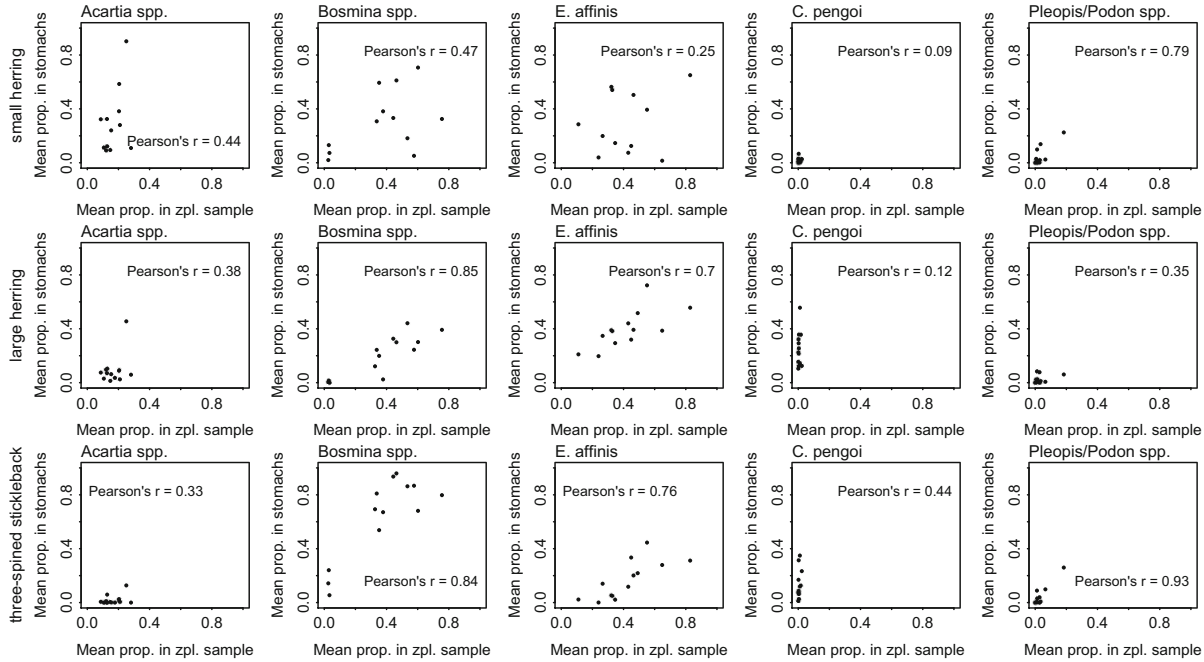

Fig. 8 Correlations between the annual mean proportion of five prey taxa in the non-empty fish stomachs (*y* axis) and in the zooplankton samples (*x* axis). Proportion in the fish stomachs is calculated as biomass share, while the proportion is zooplankton samples is based on abundance

Small-sized estuarine copepods and cladocerans dominated in the diet of both fish species. *Bosmina* spp. was the (co-)dominating prey item in the diet of all three fish groups studied, with *E. affinis* and *C. pengoi* contributing substantially to the diet of large herring and *Acartia* spp. to small herring. There seemed to be a general positive correlation between the proportion of the dominating prey (*Bosmina* spp. and *E. affinis*) in stomachs of three-spined sticklebacks and adult herring, and in the field. The diet of small herring, measured as taxonomic diversity per meal, appeared to be the most diverse, while three-spined stickleback mostly relied on one prey taxon per meal. Weak spatial patterns were detected, likely related to spatial heterogeneity in feeding habitat and species-specific preferences. It should be noted, however, that these results were obtained during the two decades that were characterized by generally warmer temperatures, lower salinity and more eutrophic conditions than the long-term mean conditions before the 1990s (ICES, 2014).

Prey composition

We found that herring was mostly feeding on a few small-sized zooplankton taxa which prefer warmer conditions and have successfully adapted to the brackish environment. This has not always been the case in the GoR: in summer of the 1960s and the 1970s, herring stomachs were mostly found to contain large-bodied and energy-rich pelagic and nektobenthic organisms, such as *L. macrurus, M. relicta*, *M. mixta*, *P. femorata* and *M. affinis* (e.g. Kostrichkina, 1968, 1970). Our results partly contradict the recent work carried out in the GoR (Livdāne et al., 2016), where the arctic copepod *L. macrurus* was found dominant in herring stomachs and contributed a significant proportion of the total biomass analysed. However, there is an agreement between our results and those from the other large gulf in the NE Baltic Sea (Gulf of Finland) in late summer in the early 2000s, where *Bosmina* sp. and *E. affinis* jointly formed the major portion of large herring diet (Peltonen et al., 2004). In 4236 large herring non-empty stomachs analysed here, we found *L. macrurus* in only 131 stomachs, i.e. 3% of specimens. One possible explanation to this difference could be the different time window of sampling—only the second half of July in our study, and whole season (May–October) covered by monthly samplings in Livdāne et al. (2016). It is possible that *L. macrurus* was abundant, and mainly selected by herring in early summer or autumn. Unfortunately, the seasonal distribution of *L. macrurus* in fish stomachs was not described in Livdāne et al. (2016).

Due to contrasting hydrological conditions and the associated prey field, varying sampling season and size distribution of the fish, notable differences in the stomach content were found between the GoR and the open Baltic herring (Casini et al., 2004; Möllmann et al., 2004).

Three-spined stickleback is generally considered an opportunistic feeder that adjusts its preferences according to the prey availability (Demchuk et al., 2015; see also Fig. 5c). The relatively limited and now several decades old evidence from shallow areas in the GoR pointed to a very diverse diet of the species, including, in addition to the five main zooplankton taxa found in the current work, substantial amounts of fish eggs and larvae, *N. integer*, *P. affinis*, *Gammarus* sp. and insects (Kostrichkina, 1970; Ojaveer et al., 1997). More recent work from the open Gulf of Finland (Peltonen et al., 2004) reported similar dominating taxa as found in our study (*Bosmina* sp., *E. affinis* and *C. pengoi*). Given the limited knowledge and sporadic nature of stomach content analyses of three-spined stickleback, future research of pelagic fish trophic interactions in the Baltic Sea should cover also this species in order to clarify the suggested causal relationship between the high densities of three-spined stickleback and declines in coastal piscivorous fish populations (Byström et al., 2015). In addition, of equal importance would be quantification of the impact of sticklebacks on the density of herring eggs and larvae in shallow coastal areas through predation (Kostrichkina, 1970; Kotterba et al., 2014).

In additon to the indigenous fauna, GoR zooplankton communities include a few non-native pelagic invertebrates which are being consumed by pelagic fish: the cladocerans *C. pengoi* and *Evadne anonyx* G.O. Sars, 1897, and the copepod *Acartia tonsa* Dana, 1849. The most important non-indigenous species in the context of pelagic trophic ecology is *C. pengoi*. In addition to its body size, *C. pengoi* is highly visible due to a large pigmented eye that makes them attractive for several ecologically and commercially important small pelagic fish in invaded ecosystems (e.g. Bushnoe et al., 2003; Gorokhova et al., 2004).

Even if not a preferred prey, *C. pengoi* occurs abundantly in fish stomachs due to its high densities in the environment (Storch et al., 2007). Predation on *C. pengoi* may be size specific, as the consumption by small fish may be hindered by the long caudal spine (Bushnoe et al., 2003). The second non-native cladoceran—*E. anonyx*—occurs at low densities in the GoR (Kalaus & Ojaveer, 2014) and in pelagic fish stomachs. Therefore, this species should be considered of negligible importance in terms of the overall energy intake. While *E. anonyx* has invaded the GoR in the early 1990s, small calanoid copepod *A. tonsa* is present in the GoR since the 1930s (AquaNIS, 2016). Unfortunately, representatives of the genus *Acartia* were not identified to the species level in fish stomachs. Therefore, it is impossible to estimate the exact proportion of *A. tonsa* in fish stomachs in the current work. As evidenced by the current study, the importance of *Acartia* spp. is substantial for small herring and remains low for large herring and three-spined stickleback.

Feeding intensity and fish behaviour

In our study, the share of empty stomachs was highest (19%) and stomach fullness lowest for the large herring, implying difficulties in finding food and potential food limitation. Limitation in prey resource is further evidenced by increased proportion of *Bosmina* spp. and *E. affinis* in their stomachs along with elevated prey densities in the field, especially since these taxa also yielded the highest stomach fullness when dominating in the stomach composition. Maturity-dependent feeding behaviour was earlier observed in Atlantic herring, with the lowest feeding intensity after spawning (Link & Burnett, 2001). Since in our study the large herring was caught while already in the developing stage of gonadal maturity during the mid-summer, this could not explain the lower feeding intensity observed here.

Contrary, the low share of empty stomachs in small herring and three-spined stickleback (<10%) indicates more favourable feeding conditions, including availability of suitable prey. Small herring was mostly relying either on *Bosmina* spp. alone or on a combination of two small-sized copepod taxa.

Previous studies indicated three-spined stickleback also as an opportunistic feeder (Ojaveer et al., 1997; Demtchuk et al., 2015); therefore, it was surprising that in this study it was found to feed mostly on one prey taxa. However, dominance of *Bosmina* spp. in the stomach does not necessarily indicate an active choice of one prey strategy, rather the predominant availability of *Bosmina* spp. in the prey field in the warm season (Lankov et al., 2010), which might be even more pronounced at small scales due to the vertical habitat overlap of three-spined stickleback and cladocerans, as both prefer warmer surface layer (Ojaveer et al., 1997, 1998).

Food consumption of small pelagic fish may be higher for those fish feeding on larger prey, due to lower handling time (van Deurs et al., 2014). This pattern was not found for large herring or three-spined stickleback in the GoR, evidenced by a broadly similar (herring) or substantially higher (three-spined stickleback) stomach fullness values when consuming smaller (*Bosmina* spp.) than larger prey (*E. affinis* and *Acartia* spp.; Fig. 5). Herring and three-spined stickleback, as visual feeders, prey actively on cladocerans due to high visibility/body pigmentation (Zaret & Kerfoot, 1975) and less evasive behaviour (Drenner, 1978). Copepods are usually selected because of their larger size (Viitasalo et al., 2001). For three-spined stickleback, it is also important to succeed at the first predation attempt—sucking prey as a whole, in one movement—otherwise the prey may be missed altogether (Visser, 1982). The latter should explain the high proportion of low-motile prey (i.e. *Bosmina* spp.) in three-spined stickleback stomachs, which rapidly increases along with prey availability in the field (Fig. 8).

Size-specific feeding

Herring exhibits pronounced size-specific feeding in the Baltic Sea (Casini et al., 2004). This was also the case in the current study, where we have found that proportion of all small-sized cladocerans and *Acartia* spp. gradually declined, and that of *C. pengoi* increased with size in stomachs of herring (Fig. 3). Predation on similar prey, *Bythotrephes longimanus* (Leydig, 1860), by rainbow smelt *Osmerus mordax* (Mitchill, 1814) in Lake Erie could achieve 66–155% greater growth if *B. longimanus* spines were replaced with digestible prey items. The indigestible spines may actually reduce fish growth by occupying space in the stomach while providing no nutritional value (Parker Stetter et al., 2005). Hence, predation on *C.*

pengoi does not necessarily result in increased individual growth rate of herring to a level observed in previous decades when the energy-rich native large-bodied copepod *L. macrurus* was available at high densities (Sidrevics et al., 1993; ICES, 2015). Herring switches to consume nektobentonic prey (mysids) during the autumn (Casini et al., 2004), and abundant availability of mysids may compensate for the relative energy shortage during the warm season, caused by decreased prey efficiency of *C. pengoi*.

Spatial patterns

Although statistically relatively weak, all three fish groups exhibited spatial patterns in feeding activity. The observed patterns can to some extent be explained by spatial variation in hydrographic conditions. Due to a lower hydrodynamic activity, seasonal thermocline is more pronounced in the NE part of the GoR, which results in significantly elevated zooplankton biomass in this area (Ojaveer et al., 1997) and the highest feeding success, indicated by the lowest share of empty stomachs of large herring in the current study. In contrast, the lowest share of empty stomachs of three-spined sticklebacks and small herring was found in the southern GoR, in Daugava River plume (Stipa et al., 1999), while the highest feeding intensity of both fish was associated with the area of propagation of the low-saline riverine water from the anticyclonic bulge of River Daugava plume in the SW basin (Lips et al., 2016). Increased turbidity in the river plume bulge likely facilitates the foraging success of sticklebacks (Quesenberry et al., 2007).

Practical implications

Our results have at least two practical implications for the sampling design, and assessment and management of ecosystems and fish stocks. Firstly, the observed spatial patterns in fish feeding activity, including the low explanatory power of the spatial patterns that implies large interannual variation, should be taken into account when designing sampling protocols and surveys of pelagic fish feeding studies. Secondly, if the management target is to secure high spawning stock biomass for the local herring population, the potential food competitors need also to be surveyed and evaluated. The current study indicated the three-spined stickleback as a potential food competitor of herring, especially for juvenile stages. To be able to quantify the potential effect of sticklebacks to the local herring population through the competition for food, the sticklebacks must first be subjected to the routine abundance/biomass surveys.

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Appendix 8.

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Patterns and drivers of fish community assembly in a large marine ecosystem

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ABSTRACT: The presence and survival of the species in a community depend on their abilities to maximize fitness in a given environment. The study of the processes that control survival and co-existence, termed 'assembly rules', follows various mechanisms, primarily related to biotic or abiotic factors. To determine assembly rules, ecological similarities of co-occurring species are often investigated. This can be evaluated using trait-based indices summarizing the species' niches in a given community. In order to investigate the underlying processes shaping community assembly in marine ecosystems, we investigated the patterns and drivers of fish community composition in the Baltic Sea, a semi-enclosed sea characterized by a pronounced environmental gradient. Our results showed a marked decline in species- and functional richness, largely explained by decreasing salinities. In addition, habitat complexity and oxygen were found to be significant drivers. Furthermore, we showed that the trait composition of the fish community in the western Baltic Sea is more similar than expected by random chance alone. This implies that environmental filtering, acting along the salinity gradient, is the dominant factor shaping community composition. However, community composition in the eastern part, an area beyond the steep decline in salinity, was characterized by fewer species with largely different trait characteristics, indicating that community assembly is also affected by biotic interactions. Our results add to the knowledge base of key abiotic drivers impacting marine fish communities and their vulnerability to environmental changes, a key concern for fisheries and marine ecosystem management.

KEY WORDS: Traits · Demersal fish · Baltic Sea · Assembly rule · Community · Abiotic · Competition · Functional richness · Biodiversity

INTRODUCTION

The presence and survival of the species in a community depend on their capacities to maximize their fitness in a given environment (Drake 1990). Studying the processes that control the survival and co-existence of species within a community, termed 'assembly rules', is a focal point of community ecology (Weiher & Keddy 1995, Pavoine & Bonsall 2011). The assembly rules follow various mechanisms and processes primarily related to biotic or abiotic processes (McGill et al. 2006). Biotic control, also known as 'limiting similarity', refers to interspecific competition, which prevents species with similar ecological niches from co-occurring (MacArthur & Levins 1967). Under strong biotic control, the resulting trait diversity of the community will be high, with the most abundant and co-occurring species having different traits (Mouillot et al. 2007a). In the case of strong abiotic control, known as 'environmental filtering', species that co-exist in the community are likely to share similar ecological traits that enable them to survive in the local environment (Keddy 1992, Violle et al. 2007). Thus, the resulting trait diversity of the

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community will be low (Mouillot et al. 2007a). Unlike these deterministic processes, 'neutral assembly' occurs when community composition follows a stochastic process or when limiting similarity and niche filtering processes act in synergy and cannot be distinguished from each other (Hubbell 2001).

To determine the assembly rules acting on community composition, ecological similarities of co-occurring species are often investigated (Pavoine et al. 2004, Mason et al. 2013). The ecological similarity between species can be evaluated from a number of traits, which refers to any measurable morphological, physiological or phenological feature that affects individual performance, e.g. growth, reproduction and survival (Violle et al. 2007, Mouchet et al. 2013, Mouillot et al. 2013). Therefore, each species can be described by a set of traits that determine its ecological niche. Trait-based indices summarize the trait ranges and values of all the species present in a community and therefore take into account the ecological niches of the species (Petchey & Gaston 2002, 2006). Trait values are likely to change in relation to environmental gradients or disturbance (McGill et al. 2006, Violle et al. 2012, Mouillot et al. 2013). Studies on assembly rules and trait diversity in the marine environment have primarily been conducted in estuaries (Mouillot et al. 2007a, Mouchet et al. 2013). Under such strong environmental gradients, abiotic control is the dominant process shaping community composition (Laughlin et al. 2011, Venn et al. 2011, Mason et al. 2013). Furthermore, environmental filtering is the most important driver of community composition at large spatial scales (Mouchet et al. 2013). However, biotic control on local community composition, through intraspecific competition, can become preponderant at smaller spatial scales (Jackson & Harvey 1989).

The Baltic Sea is a large, semi-enclosed sea that receives only limited input of saline and oxygen rich bottom water from the neighbouring North Sea (see Fig. 1). Consequently, the Baltic Sea is characterized by a pronounced environmental gradient illustrated by marked changes in temperature, salinity and oxygen conditions from the Kattegat to the northern Baltic proper (Leppäranta & Myrberg 2009). This environmental gradient is known to influence species distribution, as only a few marine species are able to reproduce and survive in the cold brackish conditions (Nissling 2002, Ojaveer et al. 2010). Hence, the Baltic Sea is a species-poor ecosystem (Bonsdorff 2006) with a pronounced decrease in richness along the environmental gradient, as shown for benthic macrofauna (Törnroos et al. 2015). Because of this pronounced environmental gradient, the Baltic Sea serves as an ideal marine case study to investigate the underlying processes shaping community assembly and patterns of species richness and trait diversity. In this study, we investigated the trait composition of demersal (bottom-living) fish communities along the Baltic Sea gradient using quantitative scientific trawl survey data and an extensive fish trait database. By comparing spatial patterns of species richness, functional richness and ecological similarity we shed light on the underlying drivers and mechanisms shaping community assembly in one of the large marine ecosystems of the world (Sherman & Hempel 2008).

MATERIALS AND METHODS

The Baltic International Trawl Survey (BITS)

The demersal fish dataset used in this study was extracted from the Baltic International Trawl Survey Database (DATRAS: www.datras.ices.dk). This trawl survey data covers an area from the Kattegat to the northern Baltic proper (Fig. 1). In this area, standard-

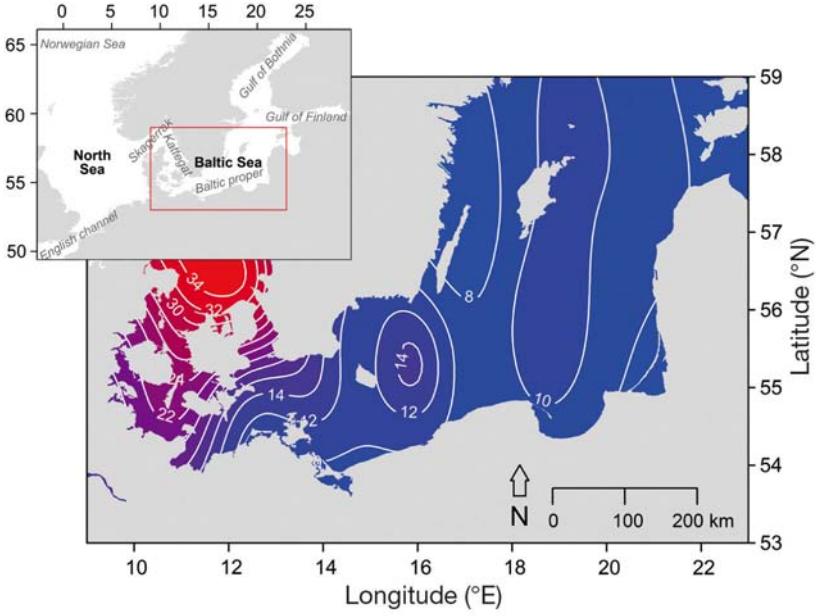

Fig. 1. Baltic Sea and its position relative to northern Europe. Colours and contour lines: pronounced bottom salinity gradient from the Kattegat to the northern Baltic proper

Table 1. Traits used to define the ecological niche of the species

Trait	Categories	Description	Relevance	Sources
Diet	Piscivorous Benthivorous Planktivorous Generalist	Main taxonomic group eaten by the species	Information on food web structure and ecological niche occupation	FishBase and literature
Age at maturity	Continuous	Age at which 50% of the population is mature	Relates to lifespan and generation time	Literature
Mean fecundity	Continuous	Average number of eggs per adult female during 1 spawning season	Relates to productivity of the species	FishBase and literature
Mean length	Continuous	Mean total length of the species in the Baltic Sea demersal survey	A proxy for many key traits, often considered most important trait in the ocean biosphere. Information on food web structure	Mean length record of the species in the BITS survey
Caudal fin shape	Rounded Truncated Emarginated Forked Continuous	The shape of the caudal fin, similar to the caudal aspect ratio but categorical	Relates to activity and habitat	Pictures
Body shape	Gadoid-like Flat Elongated Eel-like	The shape of the body, similar to the body depth/ total length ratio but categorical	Relates to habitat and position in the water column	Pictures

ized trawl surveys have been conducted annually in February or March since 1991. However, our analyses were restricted to the contemporary period (2003 to 2014) in order to avoid potential biases due to different sampling methods. At each station, all species present were sorted and recorded, and their lengths measured. When taxonomic recording was not systematically specified to species level, genus level was used. As our focus was on offshore fish communities, hauls sampled at depths <20 m were excluded from the dataset to avoid inclusion of coastal fish species. This resulted in a large database comprising approximately 3000 hauls across 70 ICES squares (1° longitude × 0.5° latitude; a standard spatial grid used for fisheries management). We determined the presence, absence and mean catch-per-unit-effort (CPUE) of species per haul and per ICES square. In order to avoid bias due to sampling effort, squares with fewer than 10 hauls were excluded from further calculation, which resulted in a total of 55 ICES squares (see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m546p239_supp.pdf). To avoid biases due to sporadically occurring species, we only included species that were present in at least one-third of all years, and present in at least 10 hauls. Using this selection criterion we retained 52 species, of which 10 were predominately pelagic. Since the catchability and abundance of pelagic species might be underrepresented in the bottom trawl survey, the analyses were conducted on the 42 demersal species only (see Table S1 in the Supplement).

Trait data

Information on 6 different traits characterizing diet, habitat and reproduction were gathered for all species included in the study. These traits are complementary as they describe different ecological aspects of the species (Table 1). For morphometric traits, pictures of each species were examined and species were assigned to different categories with respect to 'body shape' and 'caudal fin shape'. Information on diet, fecundity and age at maturity were primarily extracted from FishBase (Froese & Pauly 2012), but also verified with other sources of information (Table S1). The trait 'mean length' was calculated as the mean total length of all the individuals of a species recorded in the survey, averaged over all age classes. For the quantitative traits fecundity and age

at maturity, values were collected primarily from the Baltic Sea, but when absent, these data were gathered from neighbouring areas. For some species, trait information was not available; in these cases the data were derived from related species (Table S1).

Diversity indices

Species richness (SR) and functional richness (FRic) indices were calculated and mapped for each ICES square. SR is the number of unique species present, while FRic represents the portion of the trait space occupied by the community, defined as the convex hull volume that contains all traits present in the community (Villéger et al. 2008). FRic is based on presence–absence data, and was calculated using the FD package in R, allowing for standardization by the range of traits (Laliberté et al. 2014). Two other facets of trait diversity, namely functional evenness and functional divergence (Villéger et al. 2008), were also calculated using the same package (see Fig. S4 in the Supplement).

FRic vs. null model

Comparing the FRic of the Baltic Sea communities to values obtained from a null model can help disentangle the relative importance of the assembly rules (Mouillot et al. 2007a). The null model was constructed by calculating a mean FRic for each ICES square, obtained from a randomization process whereby species were randomly drawn from the entire species pool. The randomization matrix was obtained by controlling for both row sums (sites; i.e. ICES squares) and column sums (species) using the 'permatswap()' function in the 'vegan' package in R (Oksanen 2010). This operation was iterated 999 times. The observed FRic values were then compared to the null model and its 50 and 95% distribution. This comparison enabled a visualization of the continuum and the shifting importance of assembly rules ranging from purely environmental filtering to purely limiting similarity. We considered that observed values within the interquartile range correspond to communities where neither of the assembly rules dominated, thus indicating neutral assembly. On the other hand, observed values below or above the 25- and 75%-quartile, respectively, indicated communities predominately structured through environmental filtering or limiting similarity. Finally, we considered that communities with observed values below or above the 95% confidence intervals correspond to communities structured purely through environmental filtering or limiting similarity, respectively.

Weighted functional dissimilarity index

Assembly rules are generally investigated based on presence–absence data alone (Bellwood & Hughes 2001, Guillemot et al. 2011). However, this approach disregards important information on the relative abundance of the species considered, since species are given equal weights in the FRic calculation (Götzenberger et al. 2012). Furthermore, the limiting similarity and environmental filtering processes can also impact the abundances (May 1975, Mouillot et al. 2007b), since trait similarity may affect the degree of inter-specific competition and lead to generally lower or higher abundances under limiting similarity and environmental filtering, respectively. Therefore, we considered the abundance distribution within each community by taking into account both the relative abundance and the functional dissimilarity between each pair of species. We refer to this index as the weighted functional dissimilarity (wFDiss). To obtain a finer resolution, we calculated this index per haul instead of per ICES square. This index is similar to the Rao Q index (Rao 1982, Fig. S5 in the Supplement), but represents the mean value of the functional dissimilarity between 2 randomly selected species in the community instead of 2 randomly selected individuals. The wFDiss index is defined as:

$$\mathrm{wFDiss} = \sum_{i \neq j}^{S} \sum_{j \neq i}^{S} d_{ij} p_{ij} \quad (1)$$

with $\binom{S}{2}$ ij unique combinations, p_{ij} is the relative abundance shared by the pair of species i and j compared to the sum of the total community abundance , d_{ij} represents the functional dissimilarity between 2 species (i and j) and was assessed using a Gower distance-based analysis on the species × trait matrix (Podani 1999). The wFDiss index ranges between 0 and 1. Each pair of species can thus be characterized as having similar ($d_{ij} < 0.5$) or different ($d_{ij} > 0.5$) trait attributes. This approach is complementary to the FRic index, as it permits us to visualize if the pattern observed using presence only is also reflected when considering species abundances.

Abiotic data

To determine which environmental variables best explain the observed diversity patterns, bottom salin-

ity, temperature and oxygen data were collected for all ICES squares studied from CTD data. In addition, habitat type, habitat dominance and mean bottom depth were included as habitat-related explanatory variables and extracted from the HELCOM database (seabed sediment from the BALANCE project at http://maps.helcom.fi). Habitat type was divided into 5 categories (bedrock, hard complex, hard clay, mud and sand) and defined as the category with highest spatial coverage in each ICES square. Habitat dominance was calculated as the standard deviation of the spatial coverage of the categories in each ICES square (i.e. the higher, the less diverse is the habitat).

Statistical modelling

FRic and SR were modelled using generalized additive models (GAMs), which allow for potential non-linearities between response and explanatory variables, e.g. in case of an environmental optimum or saturation. The model equation is given by:

$$F_i \sim \alpha + \sum_{i}^{n} s(V_i) + \varepsilon_i \quad (2)$$

where F is the response variable FRic or SR, α is the intercept, s is the smoothing spline function (Wood 2006), $V_{i \to n}$ are *a priori* selected environmental explanatory variables and ε the Gaussian error term. We applied a stepwise backward selection routine based on minimizing the generalized cross validation (GCV) and likelihood ratio tests to select the most parsimonious set of predictors for each model. The s term was constrained to 3 degrees of freedom (k = 3), which allowed for second degree relationships, but restricted flexibility during model fitting. The residuals were normally distributed and spatially uncorrelated (Table S3 in the Supplement).

RESULTS

Spatial pattern of fish diversity

SR was characterized by a pronounced decrease from Kattegat (>30 species) to the northern Baltic proper (<10 species) (Fig. 2a). Rather than a gradual transition, SR showed abrupt declines at 12 and 15° E. This spatial pattern was driven by the abiotic envi-

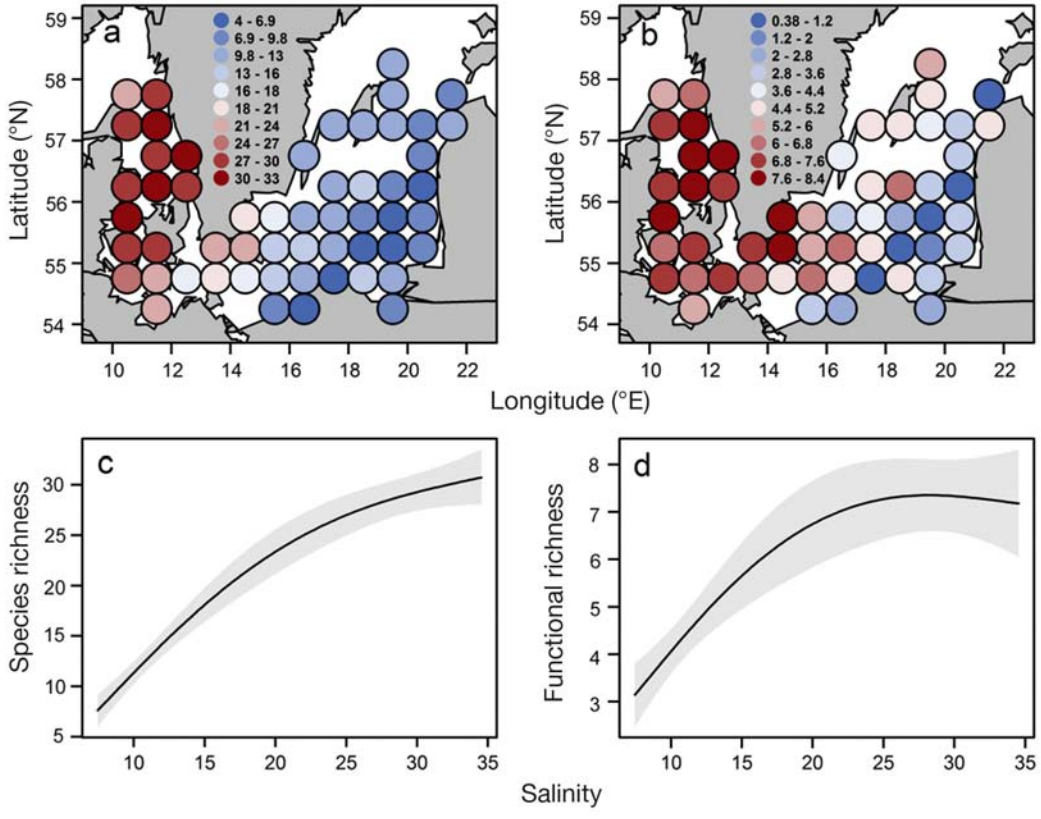

Fig. 2. Spatial patterns of fish (a) species richness and (b) functional richness and (c,d) their corresponding relationships with salinity. In (c,d): Black lines: fitted generalized additive model (GAM); grey shaded areas: SE

Table 2. Variables and parameters of the final generalized additive models (GAMs). Sign: whether the fitted relationships are predominately positive or negative; edf: estimated degrees of freedom for the model smooth terms (edf > 1 indicates a nonlinear relationship); DEV: degree of explained variance; ***p < 0.001

Best models and individual parameters	Sign	edf	DEV (%)
Species richness			
Best model: salinity + oxygen + habitat			92.5
Salinity***	+	1.91	
Oxygen***	+	0.94	
Habitat***	–	0.94	
Best single variable model: salinity			84.7
Salinity***	+	1.88	
Functional richness			
Best model: salinity + habitat			63.1
Salinity***	+	1.72	
Habitat***	–	0.87	
Best single-variable model: salinity			53.8
Salinity***	+	1.81	

ronment, especially by the pronounced salinity gradient which alone explained 84.7% of the variability (Table 2). SR displayed a positive linear response at low salinity levels until a salinity of approximately 20; thereafter the slope decreased but remained positive (Fig. 2c). Habitat dominance and oxygen showed significant negative and positive relationships, respectively, and together with salinity explained 92.5% of the variability.

FRic followed a similar decreasing pattern as SR, but remained high until 15 to 16°E despite a pronounced decrease in SR (Fig. 2b). Salinity was the best explanatory variable, explaining 53.8% of the variability. The relationship between FRic and salinity was characterized by an asymptotic relationship (Fig. 2d). Similar to SR, FRic displayed a positive linear response until a salinity level of 20, but unlike SR it reached an asymptote at salinity levels of 25. FRic had a wider confidence interval, hence a weaker relationship with salinity. Habitat dominance exhibited a significant negative relationship and together with salinity explained 63.1% of the variability.

FRic vs. null model

FRic and SR showed a saturating relationship, whereby after a certain level of SR (about 20 species), an increase in SR did not further increase FRic. The observed relationship saturated earlier than the null model and reached a lower asymptote (Fig. 3a). For low SR (<15 species), the observed FRic values were primarily distributed within the interquartile range of the null distribution. However, several communities had observed values in the upper quartile or even above the 97.5th percentile. At higher levels of SR (>25 species), FRic was largely distributed in the lower quartiles or below the 2.5th percentile of the null distribution. The observed communities with high SR but low FRic were primarily found in the western part of the study area (Katte-

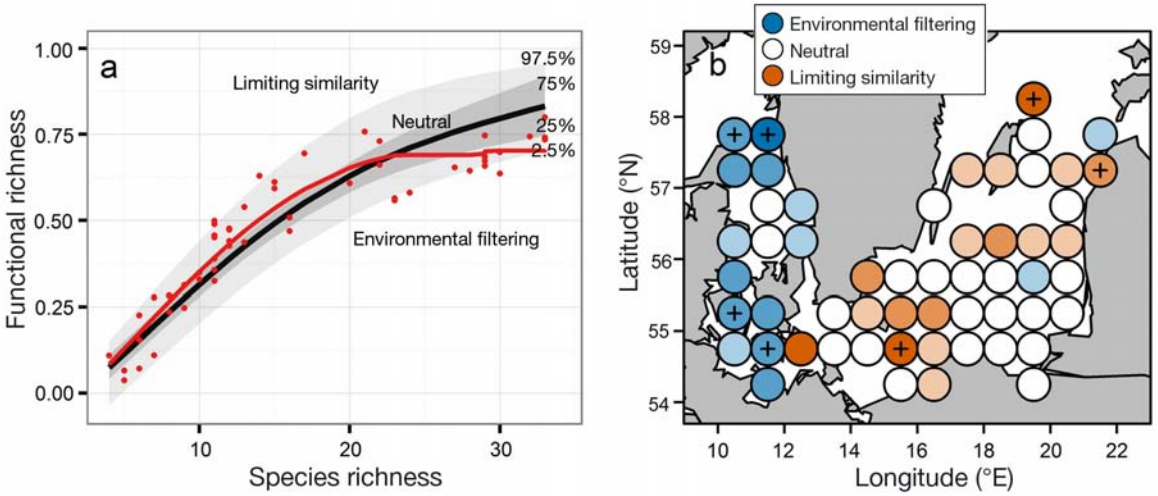

Fig. 3. (a) Observed (red dots) and simulated functional richness (FRic) values based on a null model. Bold black line: mean of 999 random permutations; areas shaded in dark and light grey: 50th and 95th percentiles, respectively, smoothed using a generalized additive model (GAM) function; red line: smooth (GAM) of the observed FRic. (b) Map of the differences (residuals) between observed and simulated FRic, in which values within the interquartile range are represented as 'neutral' assembly rules (white), while values below the 25% and above the 75% distribution are represented as 'environmental filtering' (blue) and 'limiting similarity' (orange) assembly rules, respectively. Black crosses: communities significantly different from the null model (outside the 95% range), where environmental filtering or limiting similarity alone impact community composition

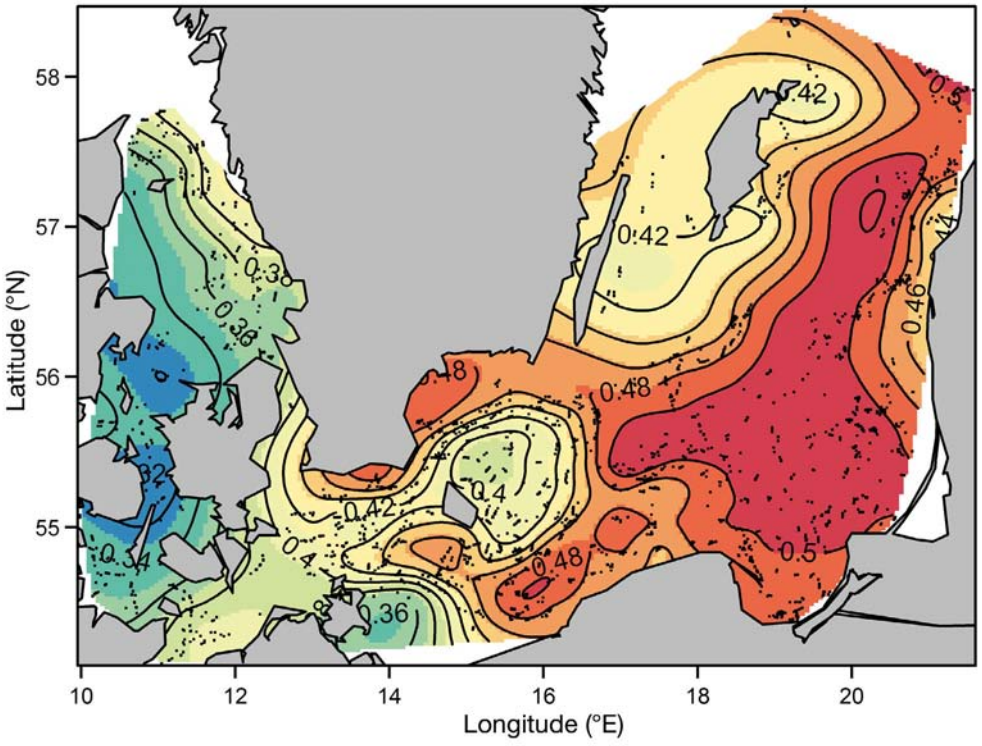

Fig. 4. Spatial patterns of weighted functional dissimilarity (wFDiss) when pairwise species abundances are considered on a per haul basis (black points), smoothed using a thin plate spline regression. Communities with low wFDiss are represented in blue; communities with comparatively higher wFDiss are represented in red. Note that the colour scheme represents only the qualitative distribution of the wFDiss values and does not imply significance

gat), while east of 14° E (Baltic proper), communities had low SR but comparatively high FRic (Fig. 3b).

Weighted functional dissimilarities of local communities

The wFDiss index demonstrates a clear spatial structure (Fig. 4). Communities with low wFDiss (i.e. with abundant species sharing similar traits) were primarily present in the western part of the region. Communities with wFDiss close to a neutral value (0.45 to 0.55) and therefore containing species having neither similar nor dissimilar trait attributes, were mainly located east of 15° E. Although wFDiss demonstrated a similar west–east pattern, it differed from the spatial patterns of FRic as its values remained low in several areas, even east of 14° E.

DISCUSSION

Environmental filtering has been shown to shape community composition along environmental gradients in terrestrial (Diaz et al. 1998, Lebrija-Trejos et al. 2010) and estuarine ecosystems (Mouillot et al. 2007a, Mouchet et al. 2013). Here, we show that environmental filtering is also the primary factor influencing fish community assembly in one of the world's large marine ecosystems, the Baltic Sea. The key underlying driver was a pronounced salinity gradient along which spatial patterns in fish community composition are structured. This is clearly illustrated by a pronounced decline in SR and FRic from maximum values in the Kattegat to minimum values in the eastern Baltic proper. This pattern is in accordance with previous studies on zoobenthos (Bonsdorff 2006, Törnroos et al. 2015) and complies with the so-called Remane's curve, which postulates species richness to be highest at salinities around 35, decreasing sharply until reaching a minimum at salinities of 6 to 8, where growth and survival of both freshwater and marine species are compromised (Remane 1934). While the salinity gradient explained most of the spatial patterns, especially in terms of SR, habitat dominance and oxygen accounted for some of the remaining variance. This means that in addition to salinity, a higher degree of bottom complexity and favourable oxygen conditions may allow for higher SR by creating more niches in terms of habitat and benthic food availability (Törnroos et al. 2013). However, a relatively high degree of variance in FRic remained unexplained (~35%), suggesting that other environmental variables not included in this study might affect the trait composition of fish communities in the Baltic Sea. Identifying these additional drivers merits further attention.

The observed relationship between FRic and SR saturated for lower SR levels than the null model. This relationship can be explained by the recurrence of particular species' traits that can be largely attributed to a number of species (e.g. *Solea solea, Scophthalmus rhombus, Melanogrammus aeglefinus, Trisopterus minutus*) present only in the western species-rich region. These primarily flatfish and gadoid species share similar traits in terms of morphology, fecundity and diet preferences. The high number of functionally similar species may therefore explain why the western communities had lower FRic values than would be expected at random. However, community composition in the eastern part of the area was characterized by a lower number of species with largely different trait characteristics. Hence, FRic values were similar or higher than expected by chance, indicating that biotic interactions may also shape community composition in these areas. Similar spatial patterns in assembly rules were also evident when using the abundance weighted wFDiss index.

The clear distinction between environmental filtering acting in the western Baltic Sea and neutral or limiting similarity acting in the east could potentially be explained by the hydrographic characteristics of the region. The western area is characterized by an unstable environment in which salinity decreases from fully marine to brackish conditions within a very short distance (approximately 400 km), whereas in the eastern part, salinity levels are low but comparatively stable, ranging from 14 in the deeper parts to 6 in shallower areas. The steep salinity gradient in the western part of the Baltic may therefore act as a filter, which would allow only species with similar key traits to survive. In the east, species poorly adapted to low salinity levels would be filtered out and the abiotic pressure would be lower, which in turn would give more room for biotic processes to impact community composition. Hence, in this area the community composition mainly follows a neutral or a limiting similarity assembly rule, possibly with both environmental filtering and limiting similarities at work.

In both areas, a few abundant and commercially exploited species (e.g. *Gadus morhua*, *Limanda limanda*, *Merlangius merlangius*, *Platichthys flesus* and *Pleuronectes platessa*) constitute most of the demersal fish abundance. Due to the commercial value of the species, fisheries could potentially impact their local abundances, and thus affect the observed patterns in wFDiss. However, fishing likely has little or no impact on the observed patterns of FRic, as the latter is based on presence–absence data only. The close resemblance between spatial patterns of assembly rules derived from both metrics, excluding (FRic) or including (wFDiss) abundances, suggest that the overall patterns are fairly robust to fishing. Nevertheless, systematic differences between the 2 indices, when accounting for the different spatial scales at which they are calculated (i.e. ICES squares vs. per hauls), may shed light on potential fishing effects.

Distinguishing which assembly rule controls community composition can provide knowledge about the biotic or abiotic drivers impacting communities, as well as provide important insight into the vulnerability of the communities to environmental changes (Keddy 1992, Mouillot et al. 2013). Functional trait diversity has a positive impact on the health and functioning of an ecosystem (Diaz & Cabido 2001, Cadotte et al. 2011, Mora et al. 2011). The study of community composition along the structuring salinity gradient of the Baltic Sea can be used to estimate how functional richness and species richness will adjust due to the predicted changes in abiotic variables under climate change. In the Baltic proper, salinity is projected to decrease by 0.5 to 5 psu, depending on the climate model scenario followed (Meier 2006). The consequences of a salinity decrease on fish communities can hence be visualized as a shift in the observed pattern with fewer marine species entering the Baltic proper. Salinity changes will likely have different impacts on SR and FRic. Due to an almost linear relationship with salinity, SR can be forecasted to change proportionally to the salinity decrease, while FRic might show only a minor decrease due to its asymptotic relationship. Nonetheless, we have shown that functional richness is already lower than expected from a null model, hence only a slight decrease could have severe consequences for the functioning and health of the ecosystem. The Baltic Sea is a complex socio-ecological system. Being bordered by 9 countries, it is subject to high anthropogenic pressures. Therefore, in an already highly impacted ecosystem, understanding the processes that structure community composition and the main abiotic drivers is of key concern for ecosystem management efforts striving to maintain a healthy and productive system, now and in the future.

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Appendix 9.

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A retrospective view of the development of the Gulf of Bothnia ecosystem

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ABSTRACT

We analysed long-term monitoring data from 1979 to 2012 in the Gulf of Bothnia, the northernmost extension of the Baltic Sea, to examine changes in the summer food web structure, and to reveal the factors causing the observed changes. Of the two sub-basins in the Gulf of Bothnia, the Bothnian Sea is more dynamic in its hydrography and food web structure than the Bothnian Bay, due to the variable influence of the more eutrophic and more saline Baltic Proper. Variation in deep-water intrusion from the main Baltic Proper, and its effect on salinity and stratification, had a clear effect on the phyto- and zooplankton communities in the Bothnian Sea. Probably due to this intrusion, the nutrient status in this basin has also changed in terms of nitrogen limitation, with subsequent class- and genus-level changes in phytoplankton community composition. The migration of cod to the Bothnian Sea during the 1980s had profound effects on the herring population, but cascading effects affecting the basis of the food web were not obvious. In contrast to a more pronounced interplay of both top-down control and bottom-up nutrient limitation of the Bothnian Sea, the Bothnian Bay food web was mostly driven by hydrography and climate, and major changes were observed in the basis of the food web. Community changes were observed in both basins in the Gulf of Bothnia throughout the entire time period. Human influence considerably affects both the basis of the food web and its very top, where man has replaced the natural top predators. Though the eutrophication status of the Gulf has been rather stable, the results indicate that the potential symptoms should be surveyed specifically in the Bothnian Sea, and that management of Baltic herring stocks requires an understanding, and thus monitoring, of the entire food web.

1. Introduction

Long-term oceanographical observations are essential for evaluating possible changes in ecosystems, following their recovery, and observing natural and/or anthropogenic causes behind ecosystem changes. Monitoring has been conducted in the Baltic Sea for decades, which enables the analysis of long-term trends in this highly dynamic ecosystem. Consistent time series data is also essential for improving models of physical-biogeochemical-ecological ocean dynamics (Ducklow et al., 2009). Recent studies of the Baltic Sea time series have discussed the potential causes for long-term changes in single ecosystem components such as phytoplankton and zooplankton (Suikkanen et al., 2013), important indicators of water quality such as Secchi depth (Dupont and Asknes, 2014), and several physical, chemical and biological parameters (Lennartz et al., 2014). All these studies reveal the strength of long-term data. However, food web analyses with physical and chemical data are scarce, as data sets of different food web compartments with similar quality are not always available. Whole-ecosystem analysis using long-term data could reveal both resource-based bottom-up effects and cascading food web effects, which act simultaneously. The subject of our study is the ecosystem of the Gulf of Bothnia in the Baltic Sea. It was selected as our target area as it is a physically highly dynamic system with high river inflow and variable winter regimes, and a number of species living at the very margins of their adaptive capability. Furthermore, the ecosystem properties of the Gulf of Bothnia are not as well-known as those of the more southern parts of the Baltic Sea.

The Gulf of Bothnia is the northernmost extension of the Baltic Sea (Fig. 1). It covers approximately 30% of the total Baltic Sea area. The Gulf of Bothnia can be considered an independent entity due to the combination of its specific characteristics. It is the most Arctic of all Baltic Sea subareas because of its northern location, and the least marine due to its distance from the Danish Straits, i.e. the connection to the North Sea and high riverine freshwater inflow. The two sub-basins of the Gulf of Bothnia, the northern Bothnian Bay and the southern Bothnian Sea, are separated by the narrow and shallow Quark area (Fig. 1). Only a limited number of organisms have adapted to living in the harsh northern conditions, leading to low a biodiversity of macroorganisms in the Bothnian Bay specifically (Elmgren and Hill, 1997). Here, the biota is a mixture of freshwater and brackish water species.

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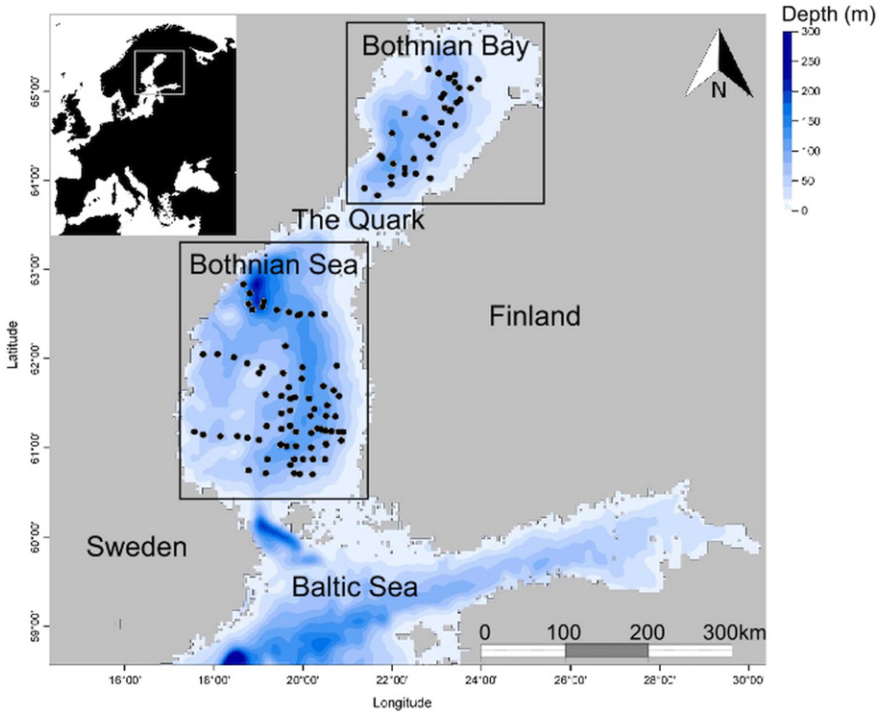

Fig. 1. Bathymetric map of the Gulf of Bothnia. The sampling stations in the Bothnian Bay and the Bothnian Sea are marked in both basins.

The species composition in the Bothnian Sea is closer to that of the Baltic Proper. However, the analysis by Elmgren and Hill (1997) shows that functionally the Bothnian Sea is similar to the Baltic Proper, and that most functional groups are also present in the Bothnian Bay though brackish water species are partly replaced by freshwater species.

The Gulf of Bothnia has no permanent salinity stratification due to southern sills, which prevent the intrusion of saline deep water into the Gulf from the Baltic Proper (Fig. 1). In the absence of a permanent halocline, deep-water layers and sediment remain nearly normoxic, which has a stabilising effect on benthic animal communities and basin-wide nutrient dynamics. The Bothnian Bay has very low algal production and it is mainly phosphorus-limited in comparison with the higher production capacity and temporal nitrogen limitation of the Bothnian Sea (Sandberg et al., 2004, Tamminen and Andersen, 2007). However, Elmgren (1984) also demonstrates a reduced flow of autochthonously produced carbon in the Bothnian Sea compared with the Baltic Proper. Terrigenous organic carbon originating from the catchment area is important as an energy and nutrient supply to the Gulf of Bothnia ecosystem, specifically in the Bothnian Bay (Sandberg et al., 2004). Consequently, the ecosystem structure in these northern Baltic basins is skewed towards bacteria-microbial loop-dominated carbon flows (Wikner and Andersson, 2012).

The variability in the Baltic Sea ecosystem is due to both human influence and natural factors. Österblom et al. (2007) showed how drastic these changes have been over the past century. They conclude that the large regime shifts, i.e. critical transitions between alternative stable states, are based on anthropogenic causes, although climate has also contributed to the changes observed. As early as the end of the 1980s, Rapport (1989), using a combination of ecosystem stress indicators including nutrients and biotic composition, found signs of 'ecosystem distress' in all spatial scales in the Gulf of Bothnia. In Rapport (1989), ecosystem distress symptoms already included early signs of eutrophication in coastal areas, a reduction in species diversity, and increased dominance by opportunistic species.

We aim to investigate changes in the Gulf of Bothnia during a 34-year period (1979–2012). In addition to the top-down control pathways (Österblom et al., 2007, Rudstam et al., 1994), we also cover the lower parts of the food web in as much detail as possible, and include hydrographic and nutrient parameters. The goals of our analysis are to examine 1) how the physical environment and nutrient status in the two basins has changed, 2) how these changes are reflected in the summer food web structure, 3) whether the changes are due to local factors or large-scale phenomena, and 4) how top-down control of the lower food web levels is compared to bottom-up limitation by nutrients.

2. Materials and methods

2.1. Study area

The Gulf of Bothnia is geographically divided into two sub-basins: the northern basin referred to as the Bothnian Bay or sometimes as the Bay of Bothnia, and the southern Bothnian Sea (Fig. 1). The Bothnian Bay and the Bothnian Sea cover areas of ca. 37,000 km^2 and 65,000 km^2, respectively. These two sub-basins are separated by the shallow (20-m) Quark, and their circulation patterns are rather isolated with only minor current systems crossing the Quark (Myrberg and Andrejev, 2006). As their hydrography and ecology define them as clearly distinguishable from each other (Myrberg and Andrejev, 2006), the two sub-basins can be analysed as separate units. A full account of the basic physics of the area is given in Leppäranta and Myrberg (2009).

Most of our data originates from regular monitoring stations in the open Gulf of Bothnia. We selected stations with appropriate monitoring data and a depth of >60 m from the Bothnian Sea (a total of 79 stations) and the Bothnian Bay (a total of 38 stations) (Fig. 1). The sampling area is very close to the combined Swedish and Finnish Exclusive Economic Zone in the Bothnian Sea, but somewhat wider than that in the Bothnian Bay. The sampling stations were expected to represent the same water mass in each area, and all available monitoring data collected was included in the analysis. Fish were sampled from the entire Gulf of Bothnia.

2.2. Data sets

Sampling period, depth, method and analyses for each parameter are given in Table 1. The time series data from 1979 to 2012 [excluding

Table 1
Sampling and analyses methods for the environmental and biological variables.

Variable	Period	Depth	Sampling method	Method/calculation	No. of samples/year (BS)	No. of samples/year (BB)
Surface water temperature and salinity	29 July–3 Sept 1979–2012	Mean 0–10 m	SBE 911plus CTD system and Rosette sampler		2–39	2–11
Deep-water temperature and salinity	Same as above	Deepest CTD measurement	SBE 911plus CTD system and Rosette sampler		2–33	2–10
Stratification index, E	Same as above	Mean 0–10 m and deepest CTD measurement	SBE 911plus CTD system and Rosette sampler	$E = [\sigma_{T(bottom)} - \sigma_{T(surface)}] * 1000 / depth_{deepest\ CTD}$, where σ_T = density (kg m^{-3}) − 1000 (Heiskanen and Kononen, 1994)	2–16	1–9
Deep-water oxygen	Same as above	Deepest measurement	Rosette sampler	Winkler technique (Grasshoff et al., 1999)	2–12	1–9
Dissolved inorganic nutrients (DIN, PO_4, SiO_4)	1 Nov–31 Dec 1978–2011	Mean 0–10 m	Rosette sampler	Spectrophotometric measurement (Grasshoff et al., 1999, Koroleff, 1979), detection limit 0.01 μM	1–9	1–19
Total nutrients (TN, TP)	29 July–3 Sept 1979–2012	Mean 0–10 m	Rosette sampler	Spectrophotometric measurement (Grasshoff et al., 1999, Koroleff, 1979), detection limit 0.01 μM	2–13	2–10
Secchi depth	Same as above		Secchi white-disk, 30 cm diameter (Fleming-Lehtinen and Laamanen, 2012)		1–27	1–10
Chlorophyll *a*	Same as above	Mean 0–10 m	Rosette sampler	Spectrofluorometric measurement (HELCOM, 1988)	2–11	1–3
Phytoplankton biomass	Same as above	Pooled sample 0–10 m	Rosette sampler, fixation with acidic Lugol's solution	Counting with inverted microscopy (Utermöhl, 1958), biomass calculation (HELCOM, 2015, Olenina et al., 2006), nomenclature follows the HELCOM PEG list v. 2012 (annually updated, available at http://helcom.fi/helcom-at-work/projects/phytoplankton)	1–2	1–2
Zooplankton abundance	Same as above	0 m–bottom	Vertical tows of WP2 net (mesh 100 μm) with a flow meter, fixation with formaldehyde	Counting with inverted microscope to the lowest taxonomic level possible (HELCOM, 1988)	1–4	1–3
Mysid abundance	Aug and Jan 1985, 1990, 2007, 2010	0 m–bottom	Vertical tows of large zooplankton net (opening 1 m, mesh size 500 μm)	Counting with stereomicroscope	32 in 1985/1991, 6 in 2007/2010	No data
Benthic fauna	May–June 1979–2012	Bottom	Van Veen grab, fixation with formalin	Counting with stereomicroscope (HELCOM, 1988)	2–20	1–13
Baltic herring	Jan–Dec 1979–2012		Sampling from trawl and trap net catches	Age determination from whole otoliths 1979–2001 and from stained otolith slice 2002–2012	NA	NA

BS = Bothnian Sea, BB = Bothnian Bay and NA = not available.

mysids (*Mysis* spp.) and Baltic herring (*Clupea harengus*)] were collected during national monitoring cruises, where sampling was conducted according to the latest version of the Manual for Marine Monitoring in the COMBINE programme of HELCOM (current version: HELCOM, 2015), which is updated annually. For environmental and plankton variables, the sampling took place mostly during August, and represents the late summer season in the study area. Exceptions to this were inorganic nutrients, which were sampled in winter, as long-term trends are most reliably detected in winter samples, and benthic fauna, which was sampled in early summer (Table 1). The data was downloaded in November 2013 from the Finnish Environment Institute and the Finnish Meteorological Institute's Sumppu marine monitoring database (nodc.fmi.fi/grafeio), from the Finnish Environment Institute's national Hertta database (www.ymparisto.fi/oiva) and from the Swedish Meteorological and Hydrological Institute's SHARK database (www.smhi.se/klimatdata/oceanografi/Havsmiljodata). Before storing monitoring data into databases, its quality is checked from the primary sources according to the quality standards of the monitoring institutes. The data used in this paper was directly extracted from the databases mentioned. Mysids were collected during monitoring cruises on board R/V Aranda in August and January 1985, 1991, 2007 and 2010. Baltic herring data originated from trawl and trap net catches (collected by the Finnish Game and Fisheries Research Institute, currently part of the Natural Resources Institute Finland). Additional herring data is from the ICES WGBFAS (Baltic Fisheries Assessment Working Group) report (ICES, 2013). It should be noted that herrings in the northern Baltic Sea are very small in size and grow slower compared to Atlantic herring populations. For example, between 1973 and 2014 the mean weight of 4-year-old specimens in the Bothnian Sea varied from 20 to 40 g. Herring is relatively evenly distributed in the Bothnian Sea, but most of the herring catch in the Bothnian Bay is caught in the south-eastern part of the area and in the northern Quark.

2.3. Variable selection

The physical and chemical variables considered included late summer surface and deep-water temperature and salinity (and density calculated from these), Secchi depth, and deep-water oxygen concentration. The deep-water sample always represented the same water mass, which is high-density near-bottom water at the depth of the deepest CTD measurement. The main nutrients (inorganic nitrogen and phosphorus and silica concentration) from the surface layer were evaluated as winter nutrient concentrations, though lack of data forced us to use early winter values instead of the whole winter period. Total nitrogen and phosphorus concentrations from the surface layer were used to reflect the late summer nutrient situation. The biological parameters included surface-layer chlorophyll *a* concentration and phytoplankton composition and genus-level biomass, together with

zooplankton composition and abundance from the whole water column in late summer, and benthic animal composition and abundance in early summer (Table 1).

The ten phytoplankton classes and 52 genera or higher taxonomic units included in our analyses were consistently identified in both sub-basins throughout the analysis period, except for the phototrophic ciliate *Mesodinium rubrum*, which was only included in the phytoplankton counts since the late 1980s. Classes with <10 observations per sub-basin (consisting of Pedinophyceae and Charophyceae) were excluded. Unidentified auto- and heterotrophic flagellates and other heterotrophic genera except heterotrophic dinoflagellates were additionally excluded. Phytoplankton was analysed at the class level using trend analyses and Redundancy Analysis (RDA; see below). However, phytoplankton was considered on the genus level in the Non-metric Multidimensional Scaling (NMDS; see below), excluding genera with <5 observations, *M. rubrum* and unidentified heterotrophic nanoflagellates. Additionally, cyanobacterial colonies with cells smaller than 2 μm were grouped into Chroococcales and Cryptophytes into Cryptomonadales.

Zooplankton units chosen for the analysis represent the dominant taxa of copepods, cladocerans and rotifers in the studied areas. Copepodites and adult copepods were analysed separately due to differences in their population dynamics. The total abundance (ind m^{-3}) of the zooplankton community, as well as the abundances (ind m^{-2}) of the most common benthic animals (*Monoporeia affinis*, *Pontoporeia femorata* and *Saduria entomon)* were also included in the analysis. The following herring parameters were considered: the estimated number of 1-year-olds and biomass of the total herring stock (TSB). The average weight of herring age groups 1 to 9+, herring spawning stock biomass (SSB) and the annual herring and cod catch were used as additional information.

Due to a lack of comprehensive monitoring data on mysids, we compared mysid numbers caught during two time periods (1985/1991 and 2007/2010) in the Bothnian Sea to gain an overview of their abundance trends.

2.4. Statistical methods

Statistical analyses were performed using the R software (R Core Team, 2012). Time series (annual averages of the environmental and biological variables in all sampling stations of each basin) were analysed using generalised additive models (GAM) (R package 'mgcv', Wood, 2014). The models are rather robust against moderate deviations from normality assumption, therefore the annual data was assumed to be normally distributed. Possible autocorrelation was modelled with AR1 (autocorrelation structure with lag 1). The GAM model was fitted using a thin plate spline smoother, and the dimension of basis (k) of the smooth term was automatically selected by the computational method. Parameter k varied between the models fitted to the variables. Curves estimated with GAM and their 95% confidence intervals were plotted on the data for visualising the significant long-term variation (Figs. 2–5). Only variables with statistically significant trends ($p < 0.05$) in either the Bothnian Sea or the Bothnian Bay are shown in Figs. 2–5.

Non-metric Multidimensional Scaling (NMDS) was used to analyse changes within the phyto- and zooplankton communities by ordinating samples based on the dissimilarities in their phytoplankton and zooplankton community composition (function metaMDS, R package 'vegan', Oksanen et al., 2013). In addition, the taxa were likewise ordinated according to their occurrence in the samples. NMDS is an ordination technique that uses rank orders to collapse information from multiple dimensions, so they can be visualised and interpreted. We used the Bray-Curtis dissimilarity as the distance metric in the NMDS, and square root transformation for genus-level phytoplankton biomass (wet weight) and zooplankton abundance results. In the NMDS ordination space, the samples position themselves based on their taxon-specific biomass (phytoplankton) or abundance (zooplankton) composition, and the taxa are likewise ordinated.

To interpret changes that occurred in the entire food web, relationships between the environmental and biological variables were examined using Redundancy Analysis (RDA; R package 'vegan', Oksanen et al., 2013) with Hellinger-transformed yearly means of the response variables. Hellinger transformation is a square root of the part-of-the-total transformed variables, i.e. each observation of a variable is first divided by the total of that variable, and a square root of the result is then taken. This transformation offers a better compromise between linearity and resolution than the traditionally used chi-square distance (Legendre and Gallagher, 2001). Additionally, the Euclidean distance measure (the default in RDA) of Hellinger-transformed species data avoids a typical problem of overemphasising rare species in the analysis (Legendre and Gallagher, 2001).

The RDA was performed for two sets of variables to examine the environmental and biological factors explaining variability in phyto- and zooplankton communities, respectively: 1) Response variable: phytoplankton class-level biomasses, explanatory variables: surface temperature and salinity, stratification, nutrient concentrations, Secchi depth and zooplankton abundance; and 2) Response variable: abundance of dominant zooplankton taxa, explanatory variables: surface and deep-water temperature and salinity, stratification, deep-water oxygen concentration, Secchi depth, phytoplankton biomass, number of 1-year-old herrings and biomass of the total herring stock.

The forward selection of explanatory variables (R package 'packfor', Dray et al., 2007) with 999 permutations was used to find the most parsimonious RDA model with the lowest number of non-collinear explanatory variables accounting for as high a share as possible of the response data variation (adjusted R^2; Borcard et al., 2011). Collinearity of the explanatory variables in each separate analysis was checked from the variation inflation factors (VIF) of the variables, and variables with a VIF > 10 were removed, as suggested by Borcard et al. (2011).

The Wilcoxon rank sum test was used to compare abundances of mysids caught in 1985/1991 and 2007/2010 in the Bothnian Sea.

3. Results

3.1. Hydrography, oxygen, nutrients and Secchi

The deep water in the Bothnian Sea gradually became warmer during the 1990s–2000s (Fig. 2). The varying summertime weather caused large variability in the inter-annual temperature averages of the surface layer, but the increasing trend was significant despite the warming being relatively small (Fig. 2, Table 2).

The Bothnian Bay surface temperatures followed an almost identical significant trend to that of the Bothnian Sea (Fig. 3), but no deep layer trend was found in the water temperature.

Bottom salinity in the Bothnian Sea peaked in the late 1970s and early 1980s (Fig. 2). The salinity level declined during the 1980s, stabilised during the 1990s, and, for the deep layer, began increasing again in the 2000s. The Bothnian Bay salinity showed a significant decrease in both surface and deep-water layers (Fig. 3). The density field of the Baltic Sea is largely dictated by salinity changes. The gradually increasing salinity difference in the Bothnian Sea between the surface and deep water is reflected in the strengthened stratification, which occurred during the 1990s (Fig. 2). Since then, stratification strength has remained at a clearly higher level than during the 1980s. Late summer deep-water oxygen closely followed the changes in stratification (Fig. 2). In the Bothnian Bay, a decrease in deep-water oxygen was observed without significant changes in stratification (Fig. 3).

Winter inorganic nitrogen concentration increased fairly steadily in the Bothnian Sea during the 1980s (Fig. 2). Since 2000 it has stabilised at a relatively high level. The inorganic nitrogen concentration in the Bothnian Bay is constantly high (Fig. 3). The simultaneous increase of inorganic phosphorus (Fig. 2) and levelling of inorganic nitrogen in

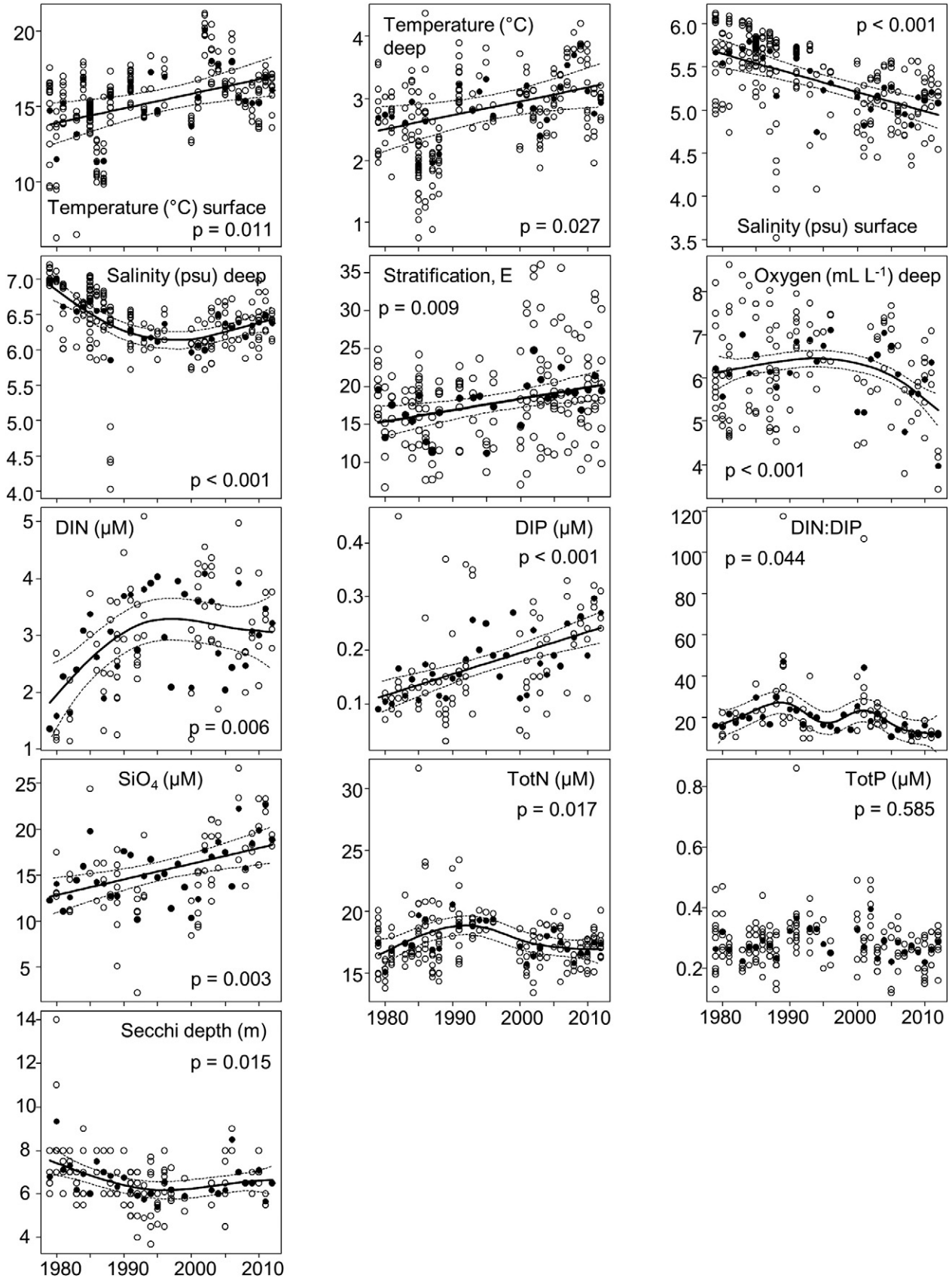

Fig. 2. Long-term trends in environmental variables in the Bothnian Sea. For variables with a statistically significant trend ($p < 0.05$), a GAM curve (solid line) is plotted with a 95% confidence interval (dashed line). Raw data is plotted as open circles and annual averages as filled circles.

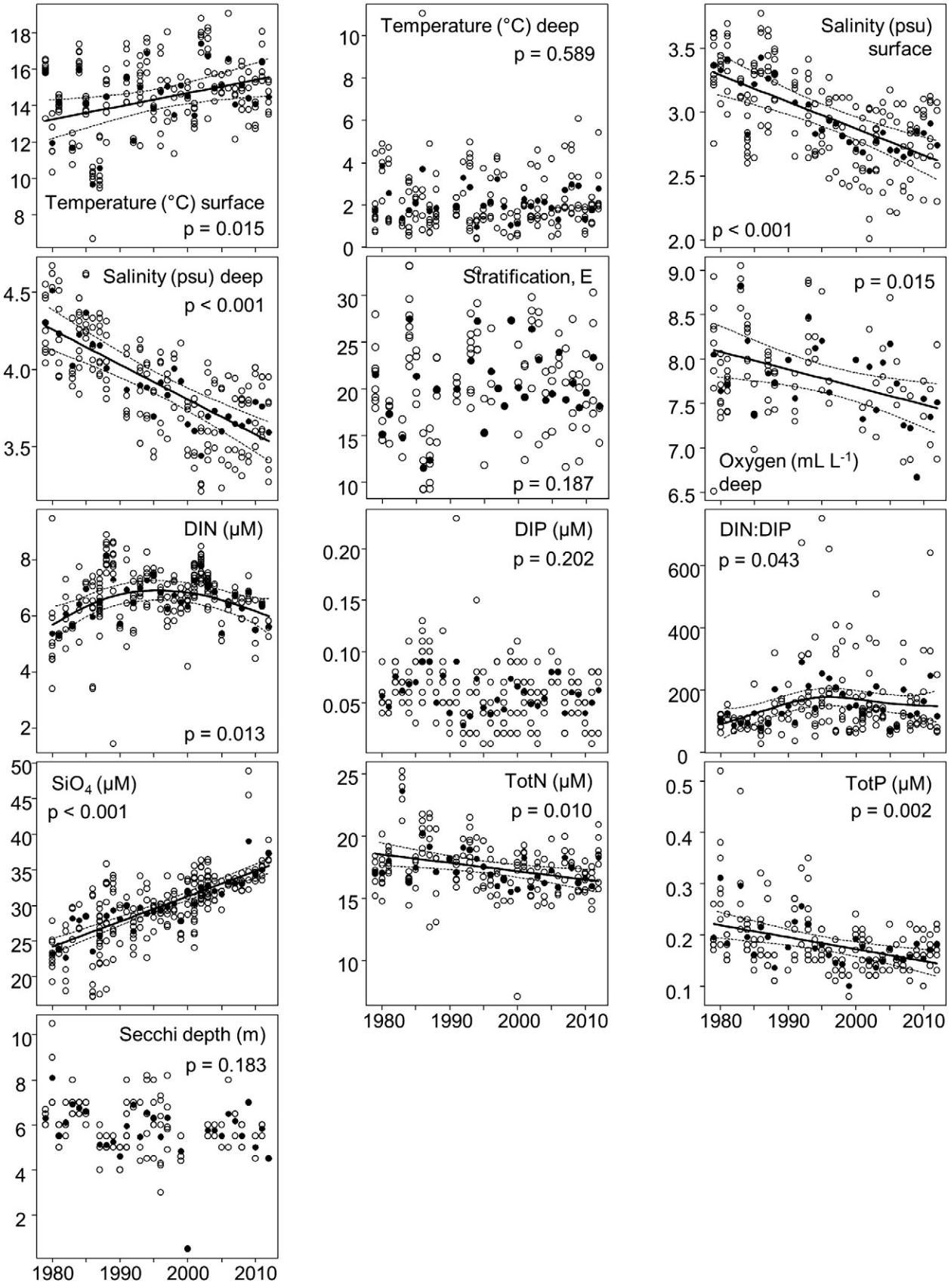

Fig. 3. Long-term trends in environmental variables in the Bothnian Bay. For more details, see Fig. 2.

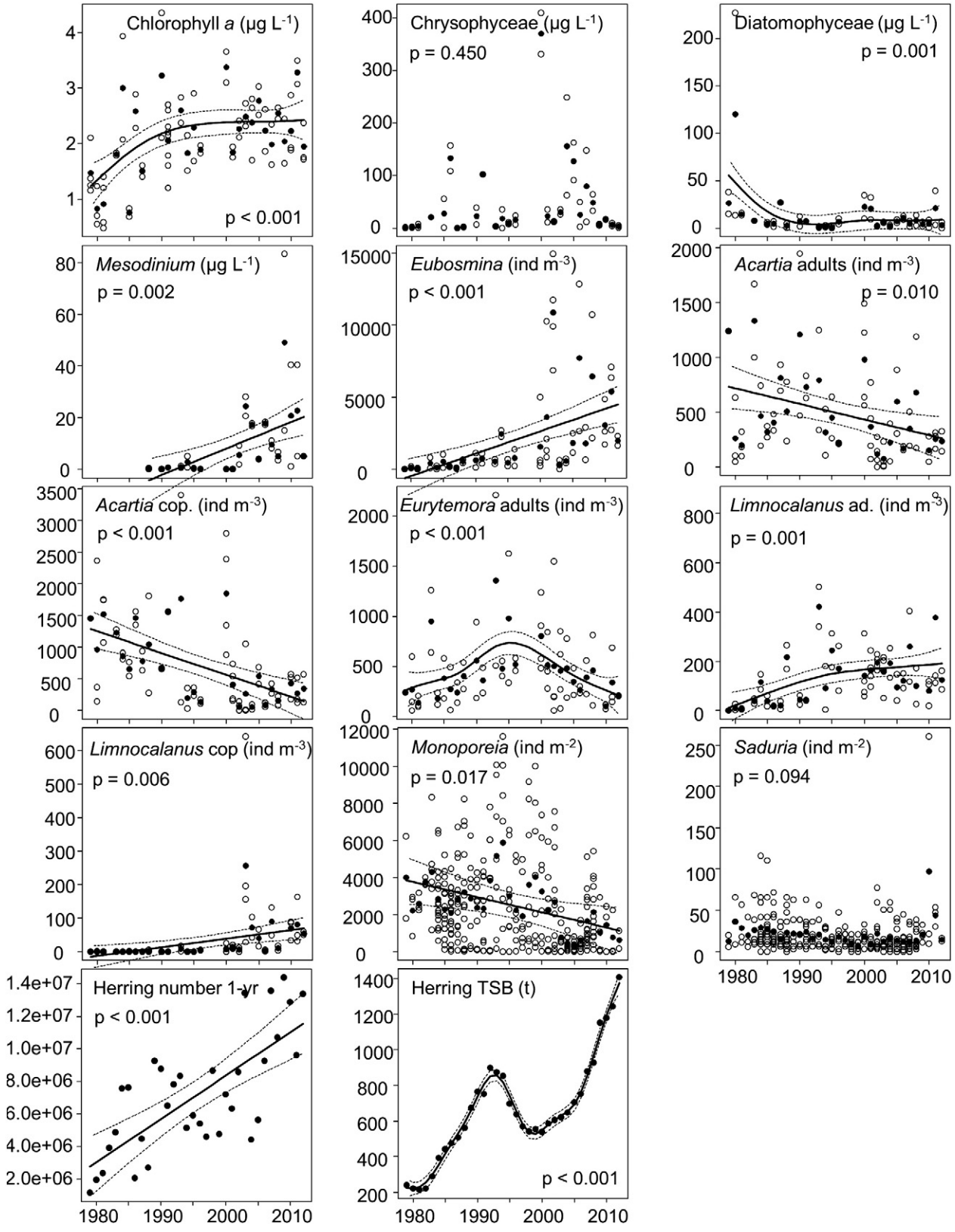

Fig. 4. Long-term trends in biological variables in the Bothnian Sea. For more details, see Fig. 2.

the Bothnian Sea resulted in a drop in the N:P ratio close to or below the Redfield ratio, compared to the situation during the 1980s and 1990s when the Redfield ratio was exceeded. The Bothnian Bay is very phosphorus-limited with an extremely high N:P ratio (Fig. 3). Silicate increased linearly in both basins (Figs. 2 and 3). There was a significant non-linear trend in late summer total nitrogen in the Bothnian Sea, with the maximum in the middle of the study period (Fig. 2). The trend for both total nitrogen and phosphorus was decreasing in the Bothnian Bay (Fig. 3). Summer Secchi depth decreased significantly during the study period in the Bothnian Sea (Fig. 2).

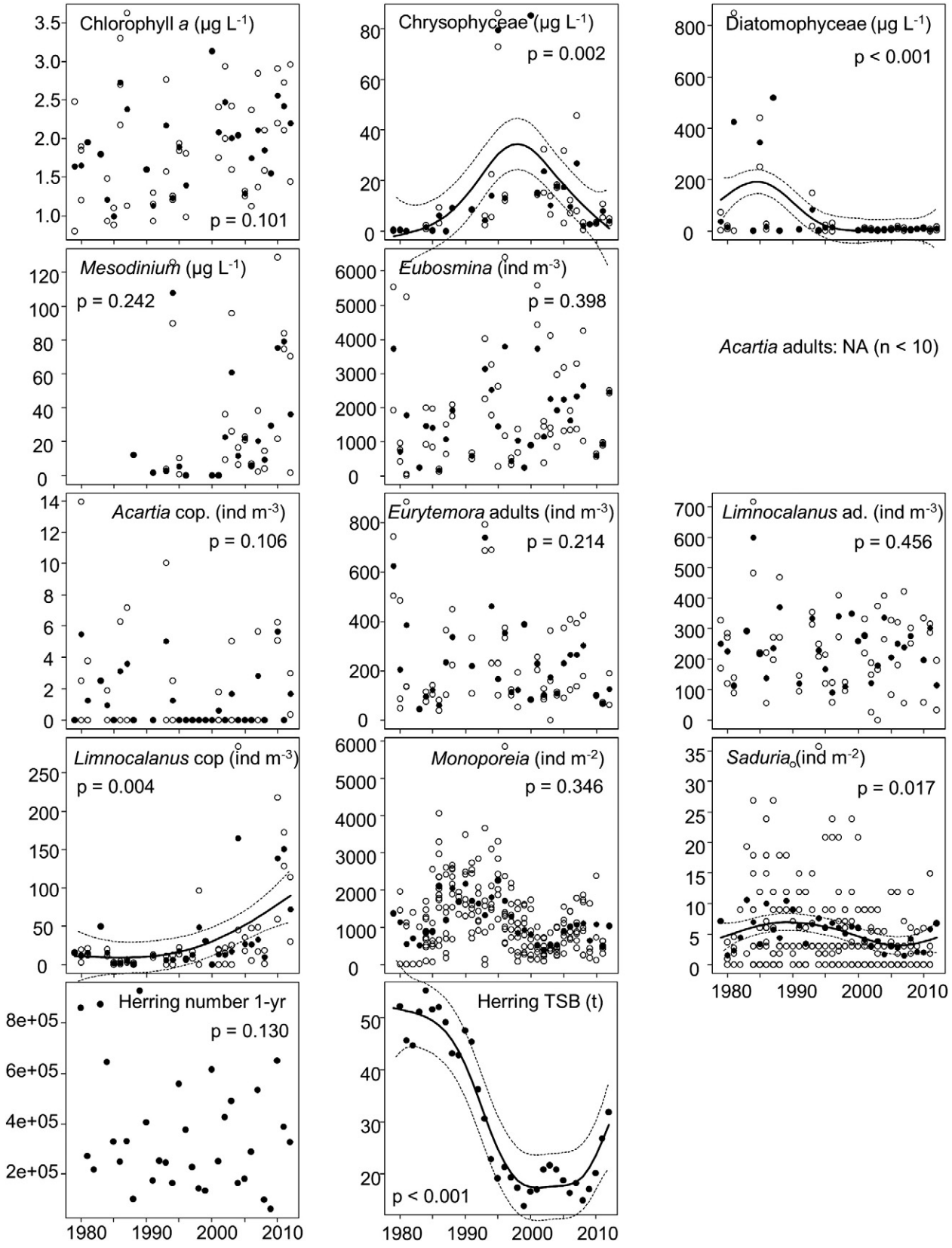

Fig. 5. Long-term trends in biological variables in the Bothnian Bay. For more details, see Fig. 2.

3.2. Phytoplankton

Significant long-term increases in chlorophyll *a* concentration (mainly in the 1980s, followed by stabilisation) and the biomass of the ciliate *Mesodinium rubrum* (from 1988 to 2012) were observed in the Bothnian Sea (Table 2, Fig. 4). The biomass of diatoms showed a significant decreasing trend. Contrastingly, no trend in chlorophyll *a* concentration was observed in the Bothnian Bay, but the biomass of

Table 2
Results of the GAM models for the detection of long-term trends.

Variable	Bothnian Sea		Bothnian Bay	
	p	*n*	*p*	*n*
Surface temperature	0.011	27	0.015	31
Deep temperature	0.027	27	0.589	31
Surface salinity	<0.001	27	<0.001	30
Deep salinity	<0.001	27	<0.001	30
Stratification	0.009	27	0.187	30
Deep oxygen	<0.001	26	0.015	27
DIN	0.006	34	0.013	33
DIP	<0.001	34	0.202	33
DIN:DIP	0.044	34	0.043	33
SiO_4	0.003	34	<0.001	33
Total nitrogen	0.017	28	0.010	32
Total phosphorus	0.585	28	0.002	32
Secchi depth	0.015	30	0.183	30
Chlorophyll *a*	<0.001	27	0.101	27
Total phytoplankton biomass	0.673	27	0.088	26
Cyanophyceae	0.432	27	0.592	26
Cryptophyceae	0.556	27	0.057	26
Dinophyceae	0.159	27	0.432	26
Prymnesiophyceae	0.668	27	0.274	26
Chrysophyceae	0.450	27	0.002	26
Diatomophyceae	0.001	27	<0.001	26
Euglenophyceae	0.472	27	0.444	26
Prasinophyceae	0.692	27	0.745	26
Chlorophyceae	0.657	27	0.828	26
Mesodinium rubrum[a]	0.002	20	0.242	19
Total zooplankton abundance	0.474	27	0.608	29
Rotifera	0.806	27	0.115	29
Daphnia cristata	NA	27	0.623	29
Eubosmina maritima	<0.001	27	0.398	29
Podonidae	0.226	27	0.083	29
Acartia spp. adults	0.010	27	NA	29
Acartia spp. copepodites	<0.001	27	0.106	29
Eurytemora spp. adults	<0.001	27	0.214	29
Eurytemora spp. copepodites	0.059	27	0.157	29
Limnocalanus macrurus adults	0.001	27	0.456	29
Limnocalanus macrurus copepodites	0.006	27	0.004	29
Monoporeia affinis	0.017	34	0.346	34
Pontoporeia femorata	0.130	34	NA	34
Saduria entomon	0.787	34	0.017	34
Herring number, 1-year-olds	<0.001	34	0.130	33
Herring, total stock biomass	<0.001	34	<0.001	33

p = significance of the GAM model, *n* = number of observations, NA = not available (<10 occurrences).
[a] Data from period 1988–2012 only.

chrysophytes and diatoms had significant, non-linear trends (Table 2, Fig. 5).

The NMDS ordination plots (Fig. 6) present the ordination of the samples based on dissimilarities in their genus-level phytoplankton community composition (left panel) and which genera characterise the samples situated in the corresponding ordination in the left panel (right panel). Based on visual inspection of the NMDS plots, the genus-level community composition of phytoplankton differed clearly between the Bothnian Sea and the Bothnian Bay (Fig. 6). However, during the study period 1979–2012, phytoplankton simultaneously shifted in the same direction in both sea areas (Fig. 6 left panel). Compared to recent years, the community in the Bothnian Bay used to be more clearly characterised by diatoms from the genus *Diatoma*, and chlorophytes from genera *Scenedesmus*, *Dictyosphaerium*, *Elakatothrix, Quadricula, Sphaerocystis* and *Monoraphidium*. Dinoflagellates from genera *Protoperidinium*, *Gyrodinium* and *Prorocentrum*, chlorophyte *Planctonema*, and chrysophytes from genera *Ollicola* and *Chrysidiastrum* were the characteristic taxa in the Bothnian Sea at the beginning of our study period (Fig. 6, right panel). Filamentous cyanobacteria from genera *Aphanizomenon* and *Nodularia* increased in importance, especially in the Bothnian Sea. For *Aphanizomenon* spp., also the absolute biomass showed a significantly increasing trend in the Bothnian Sea (S1 Fig.). Chrysophyte *Pseudopedinella,* and prasinophyte *Pseudoscourfieldia* and prymnesiophytes from genera *Chrysochromulina* sensu *lato* have also become more common in both sea areas.

3.3. Zooplankton

Cladoceran *Eubosmina maritima* increased in abundance in the Bothnian Sea (Table 2, Fig. 4), while the copepod *Acartia* spp. (both adults and copepodite stages) decreased significantly. The adults of the other two dominant copepod species, *Eurytemora* spp. and *Limnocalanus macrurus*, showed significant non-linear and increasing trends over the study period, respectively. After 2000 the copepodites of L. *macrurus* began increasing significantly (Fig. 4). The abundance of pelagic mysids, *Mysis* spp., decreased significantly during winter from the late 1980s to the late 2000s (Wilcoxon rank sum test, $W = 1$, $p = 0.002$; Fig. 7). Only a few significant changes were detected in the zooplankton community of the Bothnian Bay (Table 2). The only observed change was recorded in the copepodites of L. *macrurus,* which increased (Fig. 5).

NMDS analysis revealed significant zooplankton community changes during our research period, mainly in the Bothnian Sea (Fig. 8, left panel). The Bothnian Sea community was more characterised by *Acartia* spp. in the early years, but the composition shifted towards the Bothnian Bay community, which is dominated by cladocerans and other freshwater-tolerant zooplankton (Fig. 8, right panel).

3.4. Benthos

Significant changes were observed in benthic crustacean abundances both in the Bothnian Sea and the Bothnian Bay (Table 2, Figs. 4 and 5). Amphipod *Monoporeia affinis* showed an abundance decrease in the Bothnian Sea, whereas the abundance of isopod *Saduria entomon* saw a significant non-linear trend in the Bothnian Bay. In addition to the three crustaceans included in the analyses, the polychaete *Marenzelleria* spp. rapidly became a resident member of the benthic community towards the end of the presented time series. The first records of this species were in this dataset from 1996, and by 2005 it had established and was found in abundances reaching up to 3000 ind m^{-2}.

3.5. Baltic herring

Baltic herring spawning stock biomasses (SSB), as estimated with models in ICES (2013), differ greatly between the two basins, the Bothnian Sea having five- to seventyfold SSB compared to the Bothnian Bay (S1 Table). The difference has increased in recent years, mainly due to rapid SSB development in the Bothnian Sea. This may have increased herring predation on zooplankton and competition between herring age groups if they utilise the same food source.

In the Bothnian Sea, the average individual age group weights of the Baltic herring followed an almost identical pattern except for the first two age groups (S2 Fig.). Individual weights were above average in the period 1980 to 1994 and below that afterwards. In the Bothnian Bay, similar dynamics were present only for the last two age groups, otherwise the trend was towards leaner individuals (S3 Fig.).

3.6. Relationships between biological and environmental factors

Tri-plots of the most parsimonious RDA with phytoplankton and zooplankton as response variables are shown for both sub-basins (Fig. 9). The selected RDA model was significant ($p < 0.05$) in all cases. Adjusted R^2 (R^2_{adj}, unbiased amount of variance of the response data explained by the selected explanatory variables; Borcard et al., 2011) varied from 17.0% to 36.3% (Fig. 9).

Surface water salinity was identified as one of the most significant factors explaining variability in both phytoplankton (in the Bothnian Bay) and zooplankton communities (in both the Bothnian Sea and the Bothnian Bay) (Table 3). Stratification index E was further the most

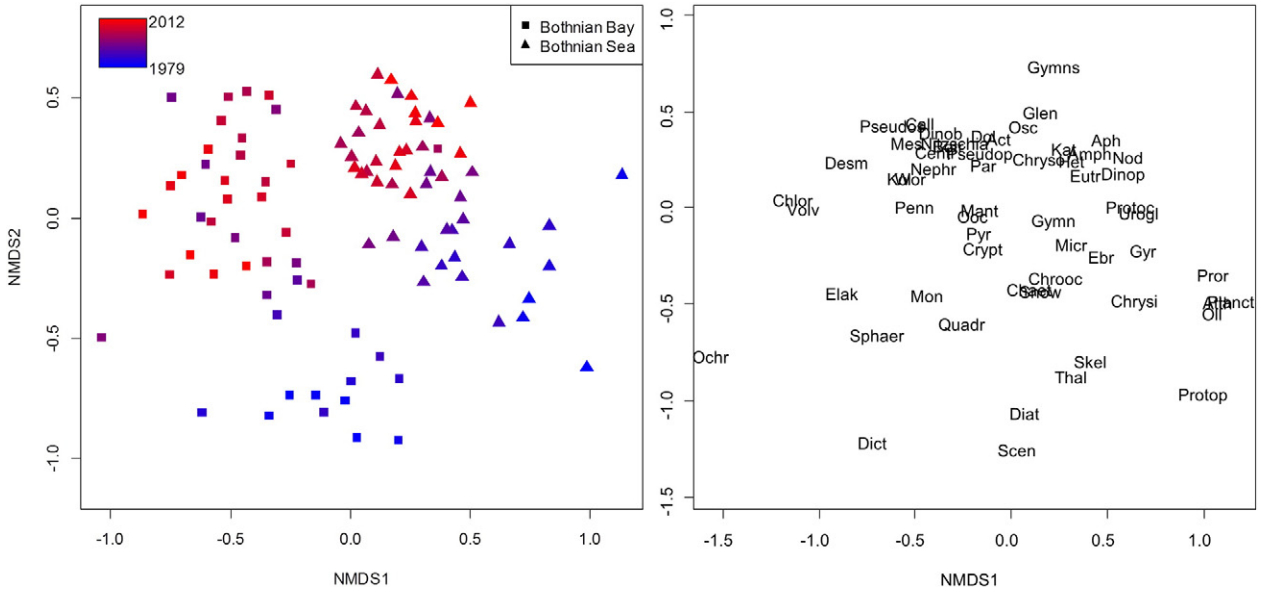

Fig. 6. NMDS based on phytoplankton biomass composition in the Bothnian Sea and the Bothnian Bay 1979–2012. Left panel: samples in the ordination plot, right panel: genera characterising the samples (Act = *Actinocyclus*, Amph = *Amphidinium*, Aph = *Aphanizomenon*, Atth = *Attheya*, Botr = *Botryococcus*, Call = *Calliacantha*, Centr = Centrales, Chaet = *Chaetoceros*, Chlor = Chlorococcales, Chrooc = Chroococcales, Chryso = *Chrysochromulina*, Chrysi = *Chrysidiastrum*, Crypt = Cryptomonadales, Desm = *Desmodesmus*, Diat = *Diatoma*, Dict = *Dictyosphaerium*, Dinop = *Dinophysis*, Dinob = *Dinobryon*, Dol = *Dolichospermum*, Ebr = *Ebria*, Elak = *Elakatothrix*, Eutr = *Eutreptiella*, Glen = *Glenodinium*, Gymn = *Gymnodinium*, Gymns = Gymnodiniales, Gyr = *Gyrodinium*, Het = *Heterocapsa*, Kat = *Katodinium*, Kol = *Koliella*, Mant = *Mantoniella*, Mes = *Mesodinium*, Micr = *Micromonas*, Mon = *Monoraphidium*, Nephr = *Nephroselmis*, Nitzsch = *Nitzschia*, Nod = *Nodularia*, Ochr = *Ochromonas*, Oll = *Ollicola*, Ooc = *Oocystis*, Osc = Oscillatoriales, Par = *Paraphysomonas*, Penn = Pennales, Planct = *Planctonema*, Pror = *Prorocentrum*, Protoc = *Protoceratium*, Protop = *Protoperidinium*, Pseudop = *Pseudopedinella*, Pseudos = *Pseudoscourfieldia*, Pyr = *Pyramimonas*, Quadr = *Quadrigula*, Scen = *Scenedesmus*, Skel = *Skeletonema*, Snow = *Snowella*, Sphaer = *Sphaerocystis*, Thal = *Thalassiosira*, Urogl = *Uroglena*, Volv = Volvocales, Wor = *Woronichinia*).

important factor explaining the variability of zooplankton communities in both sub-basins. Other significant factors explaining variability in the phytoplankton communities included rotifer and *Eurytemora* copepodite abundances, as well as the total N concentration in the Bothnian Sea (Fig. 9A). Zooplankton community composition was also significantly explained by surface water temperature in the Bothnian Sea (Fig. 9B), and by the biomass of cyanobacteria and dinoflagellates and chlorophyll *a* concentration in the Bothnian Bay (Fig. 9D).

Changes in plankton taxa were visually evaluated according to their association with the environmental variables (Fig. 9). In the RDA plot, acute angles between two vectors of either explanatory or response variables indicate a high positive correlation. None of the variables significantly explaining the phytoplankton variability in the RDA (rotifers, *Eurytemora* copepodites, total N concentration) of the Bothnian Sea expressed significant monotonic long-term trends, which makes it

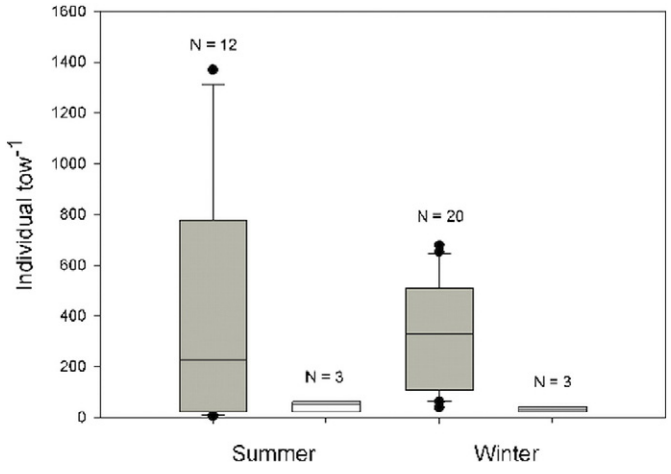

Fig. 7. Mysid abundances in the Bothnian Sea during summer and winter. Grey = 1985 and 1991, white = 2007 and 2010.

difficult to draw conclusions about their possible association with the phytoplankton classes (Fig. 9A). However, chlorophyll *a* concentration, which had a significant increasing trend, was associated with high total N concentrations and high numbers of *Eurytemora* copepodites. Total N concentration, which had a significant non-linear trend, also correlated negatively in the RDA ordination plot with Diatomophyceae, which showed a significant negative trend.

The Bothnian Bay phytoplankton community (Fig. 9C) was linked with only one significant explanatory variable: surface water salinity. The biomass of Chrysophyceae with a significant non-linear trend correlated negatively with salinity, whereas Diatomophyceae biomass (with a negative trend) was positively associated with salinity.

The significant trends in increased stratification and surface temperature and decreased surface salinity were the most important factors explaining variability in the zooplankton community of the Bothnian Sea (Fig. 9B). The cladoceran *Eubosmina maritima* and copepod *Limnocalanus macrurus*, which also expressed significant increasing trends, were positively associated with stratification and temperature and negatively with salinity. The copepod *Acartia* spp., with a significant decreasing trend, correlated positively with salinity, and negatively with stratification and temperature.

In the Bothnian Bay zooplankton community (Fig. 9D), only *Limnocalanus macrurus* copepodites expressed a significant increasing trend, which was only slightly negatively correlated with decreased surface salinity and positively correlated with Cyanophyceae biomass, but not very clearly with any other significant explanatory variable (stratification, Dinophyceae and chlorophyll *a* concentration).

4. Discussion

4.1. Trends in hydrography, oxygen and nutrients

It is inherently understood that no area in the Baltic Sea can be considered an independent unit, including the Gulf of Bothnia. The general increasing trend in water temperature is observed throughout the

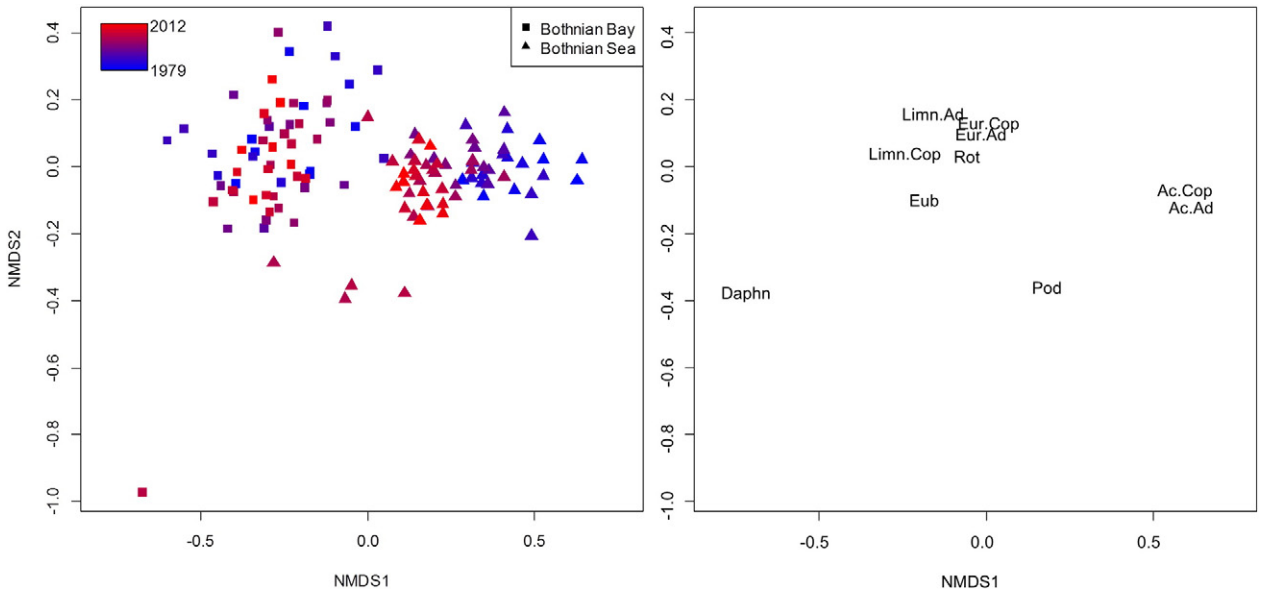

Fig. 8. NMDS based on zooplankton abundance in the Bothnian Sea and Bothnian Bay 1979–2012. Left panel: samples in the ordination plot, right panel: genera characterising the samples (Ac.Ad = *Acartia* adults, Ac·Cop = *Acartia* copepodites, Eur.Ad = *Eurytemora* adults, Eur.Cop = *Eurytemora* copepodites, Limn.Ad = *Limnocalanus* adults, Limn.Cop = *Limnocalanus* copepodites, Daphn = *Daphnia*, Eub = *Eubosmina*, Pod = Podonidae, Rot = Rotifera).

whole Baltic Sea as part of the ongoing climate change in the northern hemisphere (Heino et al., 2008). The annual mean air temperature in Finland has increased, but summer air temperature (June–August) shows more variability with a depression at the beginning of our research period, and high but historically unexceptional values after 1990 (Tietäväinen et al., 2010). Summer temperatures are decreasing again (Tietäväinen et al., 2010), as are the surface temperatures of the Bothnian Sea. This is in accordance with the temperature change across the whole Baltic Sea, with a linear increase in springtime and higher variability in summer (Heino et al., 2008).

The salinity dynamics of the 100-year time series (ending in year 2000) by Fonselius and Valderrama (2003) show that our series begins at the peak of a high deep-water salinity period (in relation to the long-term average) in the main Baltic basin. Accordingly, the salinity of the Bothnian Sea declined during the 1980s, levelled off during the 1990s, but, in the deep layer, began increasing again during the 2000s (Fig. 2). High saline water inflows to the Baltic Sea have not been specifically strong (Mohrholz et al., 2015), and they do not explain the recent rising trend of deep-water salinity in the Bothnian Sea. The salinity decline was also observed in the Bothnian Bay, where the inflow from the most northern rivers saw a positive trend from 1975 to 2010 (Räike et al., 2012), further explaining the salinity decline.

Regarding deep-water oxygen development in the Bothnian Sea, after around 2000 deep-water oxygen concentrations declined at a steady rate (Fig. 2). The oxygen development in the deep water of the Bothnian Bay followed that of the Bothnian Sea, but with a more moderate negative trend (Fig. 3). Deep-water oxygen dynamics is related to several simultaneous processes. According to the salinity trend, a larger amount of Baltic Proper water has entered the deep layers of the Gulf of Bothnia since 2000. It is a consequence of Baltic Sea-level dynamics, which has increased deep-water inflow to the basin (Rolff and Elfwing, 2015). This water may have had sub-saturated oxygen conditions, which has a direct effect on deep-water oxygen in the Gulf of Bothnia. The amount and quality of the settling material is another player in deep-water oxygen dynamics, as increased organic settling generally causes increased oxygen demand in sediments. An increase in the deep-water temperature may also have led to enhanced microbial decomposition in the Bothnian Sea. However, as we have no settling rate data for organic matter or sediment respiration measurements, their roles remain unstudied. The strengthened stratification in the Bothnian Sea may have further reduced oxygen supply to the deep water by restricting vertical exchange processes.

The decreasing deep-water oxygen level may affect the food web in phases. Even at moderate oxygen levels, higher organisms like fish may suffer from oxygen deficiency, and change their behaviour. A limit of 4 ml l^{-1} has been given for sensitive species. Cold-water zooplankton such as the important copepod *Limnocalanus macrurus* and mysids are unable to escape to the surface water, and their response is related to their hypoxia tolerance. Zoobenthos communities also change according to the sediment oxygen concentration, and finally, nutrient dynamics is heavily affected in hypoxic and anoxic sediments. Thus we should carefully follow the deep-water and sediment oxygen development in the Gulf of Bothnia.

The nutrient input from the North Sea is of minor importance in the overall nutrient balance of the Baltic Sea (e.g. Håkanson, 2009), and thus the main sources of phosphorus and nitrogen are in the Baltic Sea drainage basin. Atmospheric load is also a considerable source of nitrogen. The release of phosphorus from low oxygenated sediments is an important factor on phosphorus status in the Baltic Proper (Vahtera et al., 2007). It has counterbalanced the decline in phosphorus loads (Gustafsson et al., 2012). Currently, the good oxygen conditions in the sediment surface of the Gulf of Bothnia prevent the release of phosphorus to a large degree (Stigebrandt et al., 2014). The positive winter DIP trend in the Bothnian Sea most probably reflects both the intrusion of low-oxygen/high-phosphorus water mass from the Baltic Proper (Rolff and Elfwing, 2015) and the increasing input from the drainage basin.

The increase in winter concentrations of inorganic nitrogen in both sub-basins and its stabilisation (the Bothnian Sea) or decline (the Bothnian Bay) during the 1980s is a product of a complicated system with poorly known loss processes. External nitrogen load is still at a high level (HELCOM, 2009), but more information is needed on the effect of coastal processes on the fate of land-based nitrogen loading, for example. In the Bothnian Sea, differing trends in inorganic phosphorus and nitrogen have led to a decreasing N:P ratio, even falling below the Redfield ratio. The drainage basin is the main source of silicate, and the continuous increase of its winter concentrations is either a sign of increased silicate input or decreased uptake by diatoms. The changes in summer diatoms are not large enough to explain silicate dynamics, but we do not have conclusive data on spring bloom, with spring being the major diatom biomass period.

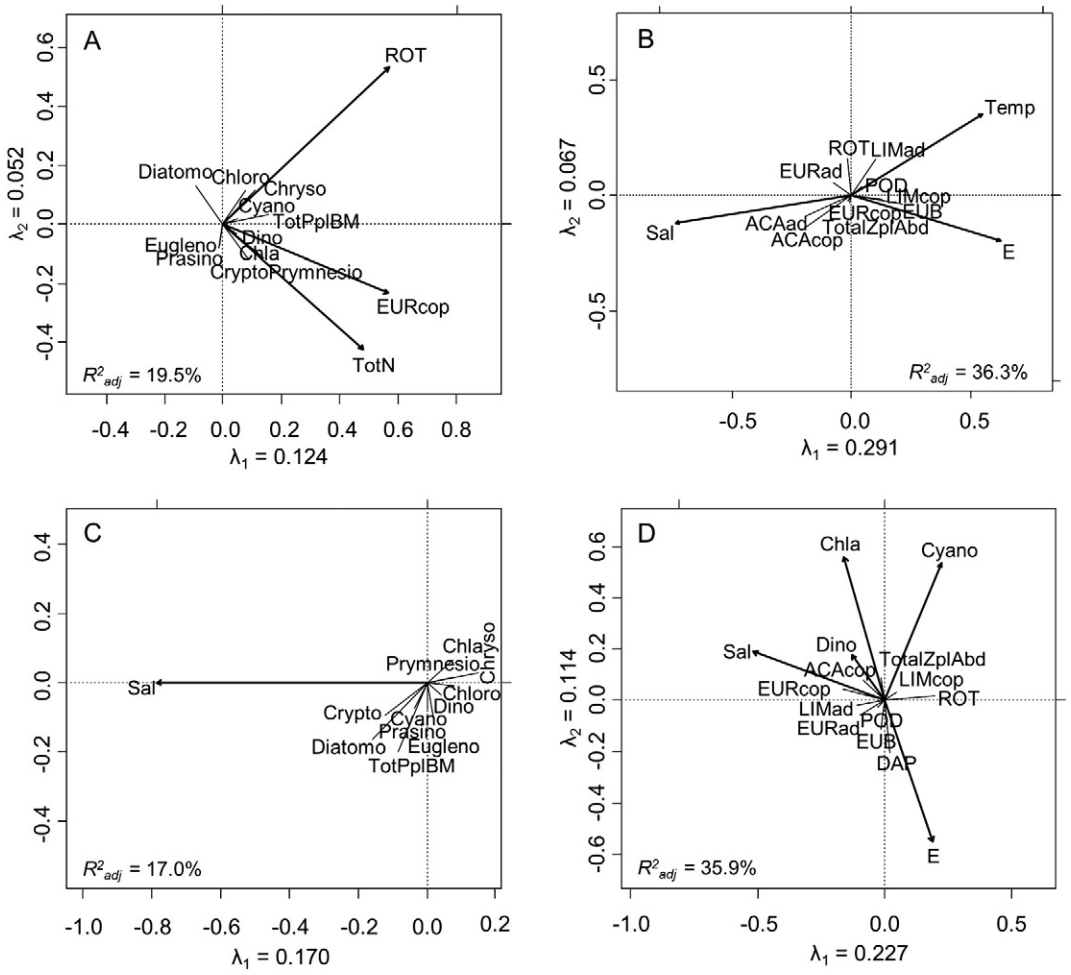

Fig. 9. RDA correlation plots for the Bothnian Sea (A and B) and the Bothnian Bay (C and D). (A and C) Response variable: phytoplankton, explanatory variables: hydrography, nutrients and zooplankton. (B and D) Response variable: zooplankton, explanatory variables: hydrography, phytoplankton and herring. Eigenvalues of the first two axes are indicated by λ_1 and λ_2. E = stratification index, Sal = surface salinity, Temp = surface temperature, TotN = total nitrogen concentration, Chloro = Chlorophyceae, Chryso = Chrysophyceae, Crypto = Cryptophyceae, Cyano = Cyanophyceae, Diatomo = Diatomophyceae, Dino = Dinophyceae, Eugleno = Euglenophyceae, Prasino = Prasinophyceae, Prymnesio = Prymnesiophyceae, TotPplBM = total phytoplankton biomass, Chla = chlorophyll *a* concentration, ACAad = *Acartia* spp. adults, ACAcop = *Acartia* spp. copepodites, DAP = *Daphnia cristata*, EUB = *Eubosmina maritima*, EURad = *Eurytemora* spp. adults, EURcop = *Eurytemora* spp. copepodites, LIMad = *Limnocalanus macrurus* adults, LIMcop = *L. macrurus* copepodites, POD = Podonidae, ROT = Rotifera, TotalZplAbd = total zooplankton abundance.

4.2. Changes in biota

Summer phytoplankton communities have changed in the Bothnian Sea and the Bothnian Bay (Fig. 6). In the Bothnian Sea, chlorophyll *a* concentration has increased (Fig. 2). This may indicate increased phytoplankton biomass as a consequence of increased nutrients, specifically phosphorus availability. However, the chlorophyll *a* to phytoplankton biomass ratio is known to vary according to the species composition

Table 3
Significant explanatory variables ($p < 0.05$) in RDA runs 1 and 2, with phytoplankton biomasses and zooplankton abundances as response variables, respectively. Adjusted R^2 (R^2_{adj}) is expressed as percentage of variance of the response data explained by the significant explanatory variables. The *F* and *p*-values are for sequential tests, i.e. type I square sums.

Explanatory variable	RDA run 1						RDA run 2					
	Response variable: Phytoplankton						Response variable: Zooplankton					
	Bothnian Sea			Bothnian Bay			Bothnian Sea			Bothnian Bay		
	R^2_{adj}	*F*	*p*	R^2_{adj}	*F*	*p*	R^2_{adj}	*F*	*p*	R^2_{adj}	*F*	*p*
Surface temperature	NS	NS	NS	NS	NS	NS	5.14	3.51	0.027	NS	NS	NS
Surface salinity	NS	NS	NS	16.99	7.75	0.003	26.30	12.78	0.001	10.08	4.70	0.003
Stratification	NS	NS	NS	NS	NS	NS	4.90	3.28	0.031	3.99	2.81	0.028
Total nitrogen	4.21	2.62	0.030	NS	NS	NS	NA	NA	NA	NA	NA	NA
Chlorophyll *a*	NA	NA	NA	NA	NA	NA	NS	NS	NS	8.44	4.31	0.005
Cyanophyceae	NA	NA	NA	NA	NA	NA	NS	NS	NS	6.86	3.85	0.015
Dinophyceae	NA	NA	NA	NA	NA	NA	NS	NS	NS	6.50	3.86	0.007
Rotifera	9.06	4.29	0.002	NS	NS	NS	NA	NA	NA	NA	NA	NA
Eurytemora spp. copepodites	6.26	3.37	0.010	NS	NS	NS	NA	NA	NA	NA	NA	NA

NS = not significant, NA = not analysed (variable not included in the RDA run).

and light environment. Thus, the increased chlorophyll *a* level may be due to the change in phytoplankton composition (Fig. 6) or coloured dissolved organic material (cDOM). Data on cDOM trends in the Baltic Sea basins is unfortunately scarce (Hoikkala et al., 2014).

Although chlorophyll *a* concentration only increased in the Bothnian Sea, we observed changes in the phytoplankton community composition in both basins. This result points out that in addition to the monitoring of chlorophyll *a* or total phytoplankton biomass, phytoplankton community composition should also be monitored to be able to detect early changes in this primary producing trophic level. The increased importance of diazotrophic cyanobacteria in the phytoplankton community of the Bothnian Sea (Fig. 6, S1 Fig.) is an expected consequence of the inorganic N:P ratio declining below the Redfield. However, the uptake of molecular nitrogen is not reflected in either winter inorganic nitrogen or late summer total organic nitrogen levels. The decline of the diatom *Diatoma* in the Bothnian Bay is not due to silicate limitation because limiting concentrations were not observed. The increase in autotrophic flagellates due to eutrophication has been reported in experimental ecosystems derived from the northern Baltic Sea (Berglund et al., 2007). However, in that experiment the responsive systematic groups were different, mainly purely autotrophic flagellates, than the potentially mixotrophic chrysophytes in our data. Another controlled experiment from the same area concluded that potentially mixotrophic flagellates can thrive in a nutrient-poor situation (Samuelsson et al., 2006). Thus, the change in phytoplankton communities may be a response to the dissolved organic matter-based food web, which is known to be important in both sub-basins, especially in the Bothnian Bay (Sandberg et al., 2004). High quality time series of the dissolved organic matter status in the Gulf of Bothnia do not exist (Hoikkala et al., 2014), but the decreasing trend in Secchi depth in the Bothnian Sea may well be an indication of increased cDOM input, in addition to the observed increasing phytoplankton biomass (chlorophyll *a*) (Fleming-Lehtinen and Laamanen, 2012).

The RDA results for the phytoplankton give an indication of the major role of surface water salinity in explaining community variability (Fig. 9). There are also indications of a top-down effect on phytoplankton from zooplankton in the Bothnian Sea based on RDA (Fig. 9), but its specific effect is difficult to pinpoint without performing experimental studies on natural communities and more frequent monitoring data. On the other hand, changes in the phytoplankton community composition may have direct bottom-up effects on the higher trophic levels of the food web through the nutritional quality of phytoplankton for herbivorous zooplankton (de Bernardi and Giussani, 1990, Sopanen et al., 2008). A change in the phytoplankton community towards a more mixotrophic and "low-quality" composition has recently been demonstrated with the monitoring data from the Gulf of Finland and northern Baltic Proper (Suikkanen et al., 2013), which concluded that the food web structure in these Baltic Sea areas appears to be changing towards a more microbial, less energy-efficient food web.

Surface salinity also explains changes in summer zooplankton communities in both sub-basins, but zooplankton additionally correlated to changes in stratification (Fig. 9, index E) and surface temperature. Strong stratification and warm water is known to benefit the microphagous cladoceran *Eubosmina maritima* (Viitasalo et al., 1995), which increased in abundance in the Bothnian Sea (Fig. 4). Decreasing salinity is known to increase the cladocera to copepod ratio in the Baltic Sea (Vuorinen et al., 1998). This is supported by our findings on the decline of the copepod *Acartia* spp. and the simultaneous increase of *E. maritima* in the Bothnian Sea (Fig. 4). The genus *Acartia* is adapted to higher salinities than the other dominant copepod *Eurytemora* spp. (Viitasalo et al., 1994), whose abundances were not seen to change during the study period. The direct indications on the resource limitation of zooplankton were rather scarce. Increased predation by herring, which is found to prey effectively on L. *macrurus* (Rajasilta et al., 2014) may explain the stabilisation of the abundance of L. *macrurus* adults in the Bothnian Sea in recent years. Mysid abundances in the Bothnian Sea may also reflect the predation pressure of the herring stock. The winter-time decrease in mysids in particular may be due to increased predation pressure, as mysids and amphipods are the main food source for herring during autumn and winter.

Benthic assemblages were in constant change, with *Monoporeia affinis* declining in the Bothnian Sea (Fig. 4) and *Saduria entomon* in the Bothnian Bay (Fig. 5). It was suggested that a decline in *M. affinis* was caused by changes in the pelagic food web and the increased importance of microbial processes, leading to a lower transport of carbon to the benthic habitat (Eriksson Wiklund et al., 2008, 2009). However, in our study we did not see benthos directly linking to our explanatory parameters. The establishment of *Marenzelleria* spp. increases both structural and functional diversity in the benthic community. *Marenzelleria* spp. have positive effects on the phosphorus retention in sediments and thus, potentially mitigate eutrophication effects (Maximov et al., 2015). However, the effects on the native fauna remain unclear.

The main intermediate predator, the Baltic herring, showed remarkable stock and weight-at-age dynamics specifically in the Bothnian Sea (Figs. 4, 5, S2 Fig, S3 Fig.). Spawning stock biomasses have increased several-fold lately in this sub-basin, with a simultaneous decline of weight-at-age (S1 Table, S2 Fig.). This proves that all age groups were becoming increasingly food-limited. The Bothnian Sea herring population was not controlled by their predators or fisheries, despite increasing herring yields in the Bothnian Sea. The effective control by cod during the 1980s led to a much lower spawning stock biomass, but together with more suitable food for Baltic herring, also to much higher weights-at-age (S2 Fig.). A very similar development regarding herring was observed in the Gulf of Riga, where salinity is close to that of the Bothnian Sea and herring is similarly the dominant clupeid species (ICES, 2013).

Our hypothesis for the age group analysis was that the weights of age groups show similar variability if their growth conditions, including food sources, are similar. According to this hypothesis, age groups 1 and 2 had different growth conditions compared to all older age groups in the Bothnian Sea. The significant negative trends in weights of age groups 1 and 2 in the Bothnian Sea and all age groups except for 8 and 9+ in the Bothnian Bay (S2 Fig., S3 Fig.) possibly point at a direct connection (e.g. migration) of these herring populations. The growing conditions were clearly best at the beginning of our study period for the Bothnian Bay population, and for a decade (1985–95) for the mature Bothnian Sea herring age groups. There may be a correlation between the subsequent years, i.e. a leaner than average 1-year-old may grow to a leaner 2-year-old and so on. However, this was not clearly visible in the data, as all trends showed similar timing, though a change towards higher individual age group weights in the Bothnian Sea after 2005 started from age group 3 and was not yet visible in age groups older than 5 (S2 Fig.).

Cod migration from the very strong Baltic Sea eastern population in 1979–1985 created a distinct population in the Bothnian Sea that was unable to reproduce. This Bothnian Sea cod population was also not observed to migrate as commonly back to the Baltic Proper as the one in the Gulf of Finland. The annual cod catch in the Bothnian Sea consequently peaked in the early 1980s (S1 Table). The catch data does not directly indicate cod stock size, as the cod fishing effort increased rapidly during the 1980s. However, a cod-rich period occurred until the early 1990s and was followed by an almost cod-free period.

The estimated predation impact of large top predators on herring in the Bothnian Sea is <6000 t per year, mainly caused by seals and to a minor extent by great cormorants (ICES, 2013). However, the catch of Bothnian Sea herring fisheries reached approximately 100,000 t in 2012 (S1 Table). The large top predators in the Gulf of Bothnia include the grey seal (*Halichoerus grypus*) and the ringed seal (*Pusa hispida botnica*). Fewer than 3000 grey seals were observed in a 2010 census of the Bothnian Sea; in the Bothnian Bay, grey seal abundance is only approximately 600 individuals, while the ringed seal population is much larger (>6000 individuals in 2010; Kunnasranta, 2012). The grey seal

potentially has an effect on older herring age groups in the Bothnian Sea, but it does not have a significant effect on the total herring population (Gårdmark et al., 2012). The ringed seal diet is dominated by the three-spined stickleback (*Gasterosteus aculeatus*), Baltic herring, smelt (*Osmerus eperlanus*) and vendace (*Coregonus albula*) (Suuronen and Lehtonen, 2012), but estimates on population feeding pressure are not available. Of the other top predators, only a minor proportion of post-smolt salmon (*Salmo salar*) remain in the Bothnian Sea, with the majority migrating to their main feeding areas in the Baltic Proper (Karlsson and Karlström, 1994). The number of great cormorants (*Phalacrocorax carbo*) has increased rapidly along the eastern Bothnian Sea coast during the 2000s according to a census by the Finnish Environment Institute (www.ymparisto.fi/fi-Fi/Luonto/Lajit/Lajien_seuranta/Merimetsoseuranta). Average herring predation by great cormorants in the Bothnian Sea has been estimated at 500 to 600 t annually in recent years, which has a low impact compared to seals, with an estimated herring predation pressure of approximately 5000 t per year (ICES, 2013).

In addition to phosphorus limitation of primary productivity in the Bothnian Bay (Tamminen and Andersen, 2007), the major differences between the Bothnian Sea and the Bothnian Bay that have an effect on food webs are the different salinity regimes and the high influence of cod in the Bothnian Sea. In this respect, the Bothnian Sea resembles the Gulf of Riga ecosystem, which is also driven by cod spillover from the central Baltic Sea (Casini et al., 2012). However, there is not much evidence on cascading effects in the Bothnian Sea. The lower levels of the food web up to the zooplankton level appear to be affected by the physical environment or resource limitation on their composition and growth dynamics. Though the eutrophication status of both basins is still under control (HELCOM, 2014), the food web is driven by a number of abiotic and biotic drivers, which should be continuously monitored. The most important drivers of the food web are large-scale physical processes affecting saline water intrusions (salinity and stratification along with the phosphorus and potential migration of cod), and human effect on the top and intermediate predators. The next major change in the drivers mentioned above may be connected to the large saline water inflow to the Baltic Sea in late 2014 (Mohrholz et al., 2015). These inflows specifically modify the deep-water environment in the Baltic Proper (Eilola et al., 2014), but how that is later reflected in the Gulf of Bothnia remains to be seen.

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Appendix 10.

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Appendix 12.

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Invasive ecosystem engineers and biotic indices: Giving a wrong impression of water quality improvement?

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ABSTRACT

Benthic component of an ecosystem is considered in ecological status assessment of the key European Directives. Most of the metrics proposed for the benthic quality assessment are biodiversity based. Their robustness and applicability are widely discussed in many recent studies. However an impact of invasive alien species on biotic indices and environmental quality assessments has been largely overlooked by researchers so far. In the current study we assessed Benthic Quality Index (BQI) in a coastal ecosystem, highly affected by the invasive zebra mussel *Dreissena polymorpha*. Zebra mussel is able of modifying benthic habitats and enhancing local biodiversity. In the analyzed ecosystem it affected benthic species richness, abundance and community structure. As a result the calculated BQI values were significantly higher in the presence of zebra mussel with evident outliers in samples with particularly high zebra mussel abundances. Therefore we found that BQI determined in our study was artificially elevated providing false signal of the ecological status improvement. Based on the results presented, we suggested data correction framework that has been tested on the current dataset and proved to be effective minimizing zebra mussel impact on BQI assessment. Our experience could be applied for other coastal ecosystems invaded by the zebra mussel or any other aquatic invasive species with resembling biological traits and bioinvasion impacts.

1. Introduction

The demand for the universal biotic indicators aimed at ecological status assessment has increased with the development of the key EU Directives, focused on reduction of anthropogenic pressures, improvement of aquatic environment and preventing biodiversity loss (Borja et al., 2010, 2013; Tett et al., 2013). EU Water Framework Directive (WFD) and Marine Strategy Framework Directive (MSFD) consider a number of ecological quality parameters, both having benthic component involved (as "Macrofauna" in WFD and "Sea floor integrity" in MSFD). There is a number of biotic metrics proposed for the benthic ecological quality assessment, including (but not limited to) Infaunal Trophic Index (ITI) (Maurer et al., 1998); Benthic Index of Biotic Integrity (B-IBI) (Kerans and Karr, 1994); Azti-Marine Biotic Index (AMBI) (Borja et al., 2000); Benthic Quality Index (BQI) (Rosenberg et al., 2004); Benthic Opportunistic Polychaetes and Amphipods Index (BOPA) (Dauvin and Ruellet, 2007); Infaunal Quality Index (IQI) (Kennedy et al., 2011).

All of them are species richness based indices utilizing quantitative characteristics of benthic communities. Indices assume that bottom-dwelling fauna are sedentary enough to escape from deteriorating environmental conditions and therefore will relatively rapidly respond to human induced pressures (Pearson and Rosenberg, 1978; Borja et al., 2000; Diaz et al., 2004; Villnas and Norkko, 2011).

To be considered as appropriate for ecological status assessment an indicator should meet the following criteria: be scientifically based (Rice, 2003; Rice and Rochet, 2005; Mee et al., 2008; Niemeijer and de Groot, 2008; Elliott, 2011); ecosystem relevant and biologically important (Niemeijer and de Groot, 2008; Elliott, 2011); responsive, sensitive, specific and predictable (Rice, 2003; Rice and Rochet, 2005; Mee et al., 2008; Niemeijer and de Groot, 2008; Elliott, 2011; Kershner et al., 2011); accurate and practical in terms of measurability and cost effectiveness (Rice and Rochet, 2005; Niemeijer and de Groot, 2008; Kershner et al., 2011).

When evaluating the environmental status of marine waters the effects of chemical pollution, eutrophication, habitat destruction and overexploitation are being addressed (Olenin et al., 2011).

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Consequently, the suitability of indicators is being tested and validated predominantly in relation to those pressures. However an impact of invasive alien species (IAS) present in the considered ecosystem has been largely overlooked by researchers so far. IAS may induce multiple important alterations in the recipient ecosystem including changes in structure and distribution of native species assemblages, habitat properties, food web structure and biogeochemical processes (Elliott, 2003; Reise et al., 2006; Olenin et al., 2007; Zaiko et al., 2011). Therefore, it is likely that impacts of other stressors may be surpassed and the correspondent ecosystem responses masked (Olenin et al., 2011).

In the current study we hypothesize, that presence and impact of an invasive ecosystem engineer may significantly influence quantitative metrics of biodiversity and therefore affect the overall ecological status assessment. In order to challenge this hypothesis, we assessed the performance of Benthic Quality Index (BQI) in a coastal ecosystem, highly affected by the zebra mussel *Dreissena polymorpha*.

BQI is a widely used multimetric indicator of benthic community condition and functionality (Rosenberg et al., 2004; Fleischer et al., 2007; Fleischer and Zettler, 2009; Leonardsson et al., 2009). Although designed for application in marine areas (Borja et al., 2003; Rosenberg et al., 2004), it has proved to be suitable for areas with strong salinity gradients given that tolerance levels of species are properly adjusted and assigned for the specific area (Zettler et al., 2007). BQI is reproducible and has been tested and validated in different marine ecosystems with varying environmental conditions (e.g. Labrune et al., 2006; Fleischer et al., 2007; Fleischer and Zettler, 2009), therefore it was advised by the international expert groups (e.g. HELCOM CORESET) for distinguishing impacted habitats from undisturbed ones.

Zebra mussel is known as a powerful ecosystem engineer capable of modifying physical, morphological, biological and biogeochemical properties of the bottom habitats (Stewart et al., 1998; Karatayev et al., 2002; Minchin et al., 2002; Zaiko et al., 2009, 2010). As it was previously reported, zebra mussels are generally associated with increased benthic macrofauna abundance, species richness and decreased community evenness (Ricciardi et al., 1997; Zaiko et al., 2009; Atalah et al., 2010). Being one of the most abundant and widely distributed IAS in the oligohaline regions of the Baltic Sea (Zaiko et al., 2011; Fenske et al., 2013) zebra mussels produce dense colonies and beds of empty shells forming patches of high biodiversity and facilitating establishment of native and non-indigenous species (Zaiko et al., 2007, 2009). It has been shown recently that some eutrophication-related metrics (e.g. nutrient levels, chlorophyll concentrations, water clarity) might be affected and lose their explanatory value in ecosystems invaded by zebra mussel (Atalah et al., 2010; Zaiko et al., 2014). Therefore, in this study we test its effect on a benthic quality assessment and suggest a framework how the IAS-related bias could be minimized.

2. Materials and methods

2.1. Study area

The Curonian Lagoon is a large (1.584 km^2), shallow (average depth 3.8 m) coastal water body connected to the south-eastern Baltic Sea by the narrow (0.4–1.1 km) Klaipeda Strait (Fig. 1). The ecosystem is greatly dependent on the Nemunas river runoff (98% of the total freshwater discharge), draining substantial amount of nutrients from the basin (Zaromskis, 1996). Ongoing eutrophication is one of the most important problems in the lagoon, affecting all ecosystem components including bottom habitats (Olenina and Olenin, 2002; Olenin and Daunys, 2004; Aleksandrov, 2010).

The lagoon is oligohaline in its narrow northern part (with irregular rapid salinity fluctuations in the range of 0.5 to 5–6 PSU) and limnic in its central and southern parts (with a relatively closed water circulation and lower current velocities. Therefore these parts serve as the main depositional area of the lagoon (Olenina and Olenin, 2002; Gasiunaite et al., 2008).

D. polymorpha was probably introduced into the Curonian Lagoon in the early 1800s. The molluscs were presumably attached to timber rafts and reached the lagoon via the central European invasion corridor (Olenin et al., 1999; Fenske et al., 2013). Currently, zebra mussels are highly abundant in the Curonian Lagoon, occupying the littoral zone down to 3–4 m depth and occurring on both hard substrates and soft bottoms. The habitats affected by zebra mussel comprise nearly ¼ of the lagoon bottom area with the largest zebra mussel community located in the central part (Zaiko et al., 2009). Soft bottom devoid of zebra mussels is dominated by oligochaetes, chironomids and another IAS *Marenzelleria neglecta* (Zettler and Daunys, 2007).

2.2. Data collection

In this study, data on macrofauna abundances from the Curonian Lagoon were analyzed for BQI development and assignment of sensitivity values. We used a long-term (2000–2010) dataset of 12 sampling events at 5 permanent monitoring sites resulting in 113 benthic macrofauna samples (Fig. 1). To enhance the data resolution, additionally we included 30 macrofauna samples from 10 sampling sites surveyed in the course of comprehensive study of zebra mussel population in 2006 (Fig. 1). For the further validation of results, the developed framework was tested with a smaller dataset from the 1999 survey (32 benthic macrofauna samples). Index response to organic carbon content in sediments (as a proxy of eutrophication-related pressure) in the context of zebra mussel presence was verified on those data.

All the samples were collected using Van Veen grab with 0.1 m^2 sampling area, and analyzed following standard guidelines for bottom macrofauna sampling (HELCOM, 1988). Due to the high small-scale bottom patchiness (Olenin and Daunys, 2004; Zaiko et al., 2009), replicate grabs from one station and sampling event were not averaged for abundance and species number and considered as individual samples (as e.g. in Leonardsson et al., 2009).

To reduce the inconsistency in the taxonomic resolution of the dataset, part of the species were pooled into the higher taxonomic groups: e.g. Oligochaeta (excluding *Eiseniella tetraedra*), Chironomidae, Trichoptera, Turbellaria, Nematoda, Heteroptera, Nemertea, Gammaridae, Unionidae, *Pisidium*, *Valvata*, *Sphaerium*). Species with presence/absence data only (e.g. hydroids *Hydra vulgaris*, *Cordylophora caspia*) were not included into analysis.

2.3. Benthic Quality Index calculation

The macrofauna abundance data were used for the computation of the Benthic Quality Index (BQI) (Rosenberg et al., 2004). Since the original version of BQI is known to be sampling effort dependent (e.g. increase in sampling effort results in higher probability of obtaining rare species), the adjusted calculation was applied (Fleischer et al., 2007; Fleischer and Zettler, 2009):

$$BQI_{ES} = \left(\sum_{i=1}^{n} \left(\frac{A_i}{A_{tot}} \times ES_{50_{0.05i}} \right) \right) \times \log(ES_{50} + 1) \times \left(1 - \frac{5}{5 + A_{tot}} \right) \quad (1)$$

In the above equation, n denotes the observed species number. A_i stands for the abundance of the species i and A_{tot} is the sum

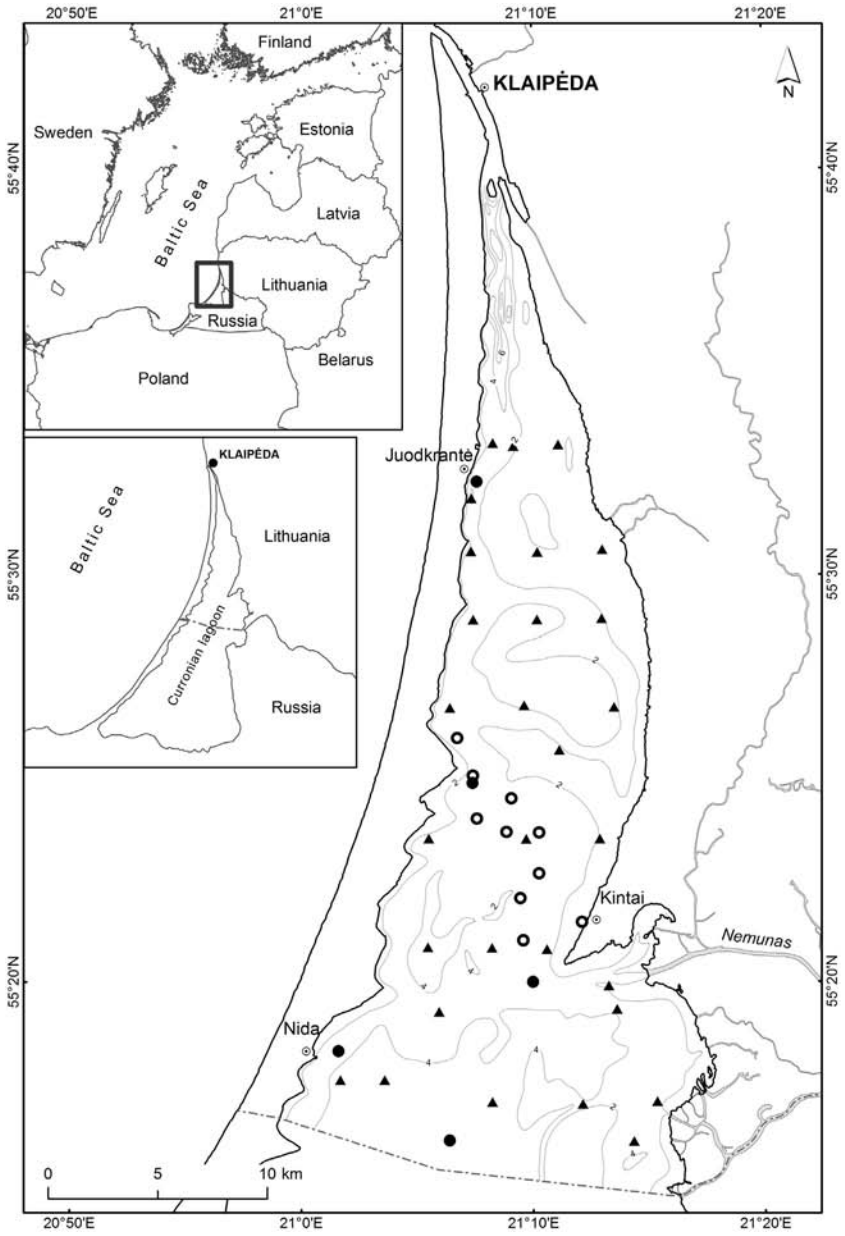

Fig. 1. Sampling sites in the Curonian Lagoon. Filled circles indicate permanent monitoring stations, open circles – survey stations in 2006, triangles - survey stations in 1999.

of all individuals within this square meter. Finally, $ES_{50-0.05}$ is the sensitivity/tolerance value for the species i and ES_{50} denotes the expected number of species for 50 individuals randomly taken from the square meter (Hurlbert Index).

The Primer software package (Clarke and Warwick, 2001) was used for calculation of the Hurlbert Index (ES_{50}). Species recorded in 10 samples or less (occurrence approx. less than 10% in our case) were excluded from further sensitivity determination, but were considered when estimating n and A_{tot} in Eq. (1) following approach used in other studies (e.g. Leonardsson et al., 2009). No samples were discarded from the analysis due to the low total abundance (less than 50 individuals) as advised by the other authors (Rosenberg et al., 2004; Puente and Diaz, 2008; Fleischer and Zettler, 2009). As originally proposed by Rosenberg et al. (2004), the sensitivity value of a species was set to the 5th percentile of the ES_{50} ($ES_{50-0.05}$). This approach follows the assumption that the most tolerant species are likely to be associated with the lowest biodiversity, lower ES_{50} values and therefore attaining lower sensitivity estimates. $ES_{50-0.05}$ was calculated as described by Leonardsson et al. (2009). Based on the estimated sensitivity values, the pre-selected species were classified by expert judgment as '*very tolerant*', '*tolerant*', '*sensitive*' and '*very sensitive*'.

2.4. Dataset correction

In order to minimize the IAS effect on the BQI assessment outcome, following correction framework was applied on the original dataset. First, the species observed only in samples with zebra mussel were eliminated in order to reduce artificially elevated ES_{50} values in locations with zebra mussels. Then, the samples with particularly high zebra mussel abundances were excluded. Since habitats dominated by zebra mussel maintain benthic communities structurally different from those observed in areas with no or low numbers of zebra mussels (Thayer et al., 1997; Strayer et al., 1998; Zaiko et al., 2009; Minchin and Zaiko, 2013), we set a threshold of zebra mussel abundance at approx. 1000 ind/m^2, corresponding to a few average size clumps which are capable to modify the soft-bottom habitats to the stage when zebra mussel-specific

communities form (Zaiko et al., 2009). Finally, abundance correction was applied for species demonstrating significant correlation with zebra mussel. Here we used a proportional correction, based on the coefficients determined in the regression model:

$$A_{i\text{-}corr} = \frac{A_i}{\beta \cdot A_{zm}} \quad (2)$$

In the above equation $A_{i\text{-}corr}$ stands for the corrected abundance of the species i, A_i – initial species abundance observed in the sample, β – slope (standardized) coefficient from the fitted linear regression model ($y = \alpha + \beta x$), A_{zm} – zebra mussel abundance in the sample (note: the correction should be applied for samples with zebra mussel only; otherwise the initial values are left).

2.5. Statistical analysis

Logarithmic transformations were applied to macrofauna abundance and organic carbon content data in order to avoid distortion resulting from the outlying values, defined during the exploratory data analysis (visual assessment of box-plots and QQ-plots).

Non-parametric Mann–Whitney (Wilcoxon) W test was used to test differences (e.g. pairwise comparisons of BQI values in samples with and without zebra mussels) when datasets were unbalanced and did not meet normality assumptions. Linear regression model with a robust fitting algorithm was applied to ascertain the effect of the zebra mussel abundance on BQI values. In case of multiple pairwise comparisons or correlation analyses (e.g. for correlations between abundances of zebra mussel and other species) the Bonferroni correction for α was applied.

Analysis of Covariance (ANCOVA) was used to verify the effects of organic carbon contents (% of sediment dry weight) on BQI values, with zebra mussel presence as a co-variate (two groups), and compare the regression slopes and intercepts between groups. Prior to that, the compliance with assumptions of homogeneity of group variances and independence of predictor variables was tested. The results confirmed that assumptions are fulfilled ($F = 2.47$; $p = 0.09$ and $F = 3.79$; $p = 0.06$ respectively).

The analyses were implemented in the R v3 statistical computing environment (R-project, 2014).

3. Results

When applying the rule of ES_{50} calculation for species occurring in ≥10 samples only, we were able to assign sensitivity values for 19 species/taxa (Table 1).

Four sensitivity classes were determined and assigned to the species using the nearest default non-decimal numbers for estimated $ES_{50-0.05}$ values: very tolerant ($ES_{50-0.05} < 2.0$); tolerant ($2.0 \leq ES_{50-0.05} < 3.0$); sensitive ($3.0 \leq ES_{50-0.05} < 4$) and very sensitive ($4 \leq ES_{50\text{-}0.05}$). There were 15 other species or higher order taxa with the occurrence ranging from less than 1% to 8%: Nemertea, Nematoda, Hemiptera, Heteroptera, Ceratopogonidae, Corophiidae, Simuliidae, *Marenzelleria* spp., *Asellus aquaticus*, *Caenis macrura*, *Glossiphonia concolor*, *Gordius aquaticus*, *Piscicola geometra*, *Potamopyrgus antipodarum*, *Radix auricularia*. These taxa were not included into sensitivity assessment.

The calculated BQI values varied between 0.54 and 2.61 with four apparent outliers in data from 2006 (Fig. 2). The highest (>2) obtained BQI values coincided with more than ten-fold elevated abundances of zebra mussels (1513 ± 1862 ind/m^2 versus 94 ± 207 ind/m^2 average in the other samples), *Pisidium* sp. (1190 ± 1425 ind/m^2 versus 42 ± 107 ind/m^2), *Valvata* spp. (592 ± 818 ind/m^2 versus 42 ± 160 ind/m^2) and Ostracoda (4852 ± 3699 ind/m^2 versus 155 ± 709 ind/m^2).

In general, BQI values in samples with zebra mussels were significantly greater ($W = 2548$, $p < 0.001$) comparing to those devoid of zebra mussels, with no apparent temporal trend (Fig. 2). Additionally, analysis of samples with presence of zebra mussels demonstrated an evident effect of *D. polymorpha* on the total macrofauna abundance (Fig. 3), significantly correlating with *E. tetraedra* ($r = 0.42$, $p = 0.002$), *Erpobdella octoculata* ($r = 0.62$, $p < 0.001$), *Helobdella stagnalis* ($r = 0.61$, $p < 0.001$), Chironomidae ($r = 0.51$, $p < 0.001$) and Trichoptera ($r = 0.53$, $p < 0.001$). On the other hand, only first three species showed significantly higher abundances (Mann–Whitney test, $p < 0.0001$) in the presence of the zebra mussels. Positive correlation was also found between the zebra mussel abundance and species richness ($r = 0.43$, $p < 0.001$). There were 3 species recorded from zebra mussel-free samples only: *G. aquaticus*, *G. concolor* and non-indigenous gastropod *P. antipodarum*. However due to their low abundances and occurrence below 5%, these species had minor effect on the estimated BQI values. Seven taxa were observed exclusively in samples with zebra mussels: *A. aquaticus*, *C. macrura*, *R. auricularia*, Ceratopogonidae, Corophiidae, Simuliidae, Hemiptera. Consequently, calculated BQI values showed statistically significant correlation with zebra mussel abundance (Fig. 4).

Table 1
Sensitivity values of the pre-selected 19 taxa, from uncorrected data analysis and suggested sensitivity class (1 – very tolerant; 2 – tolerant; 3 – sensitive; 4 – very sensitive).

Taxa	$ES_{50-0.05}$	Sensitivity class
Oligochaeta	1.4	1
Chironomidae	2.0	2
Gammaridae	2.2	2
Unionidae	2.2	2
Valvata spp.	2.4	2
Hydracarina	2.5	2
Ostracoda	2.8	2
Glossiphonia complanata	3.0	3
Eiseniella tetraedra	3.0	3
Trichoptera	3.1	3
Turbellaria	3.1	3
Helobdella stagnalis	3.2	3
Pisidium sp.	3.4	3
Dreissena polymorpha	3.5	3
Erpobdella octoculata	3.5	3
Sphaerium spp.	3.9	3
Glossiphonia heteroclita	4.0	4
Viviparus viviparus	4.1	4
Bithynia spp.	4.7	4

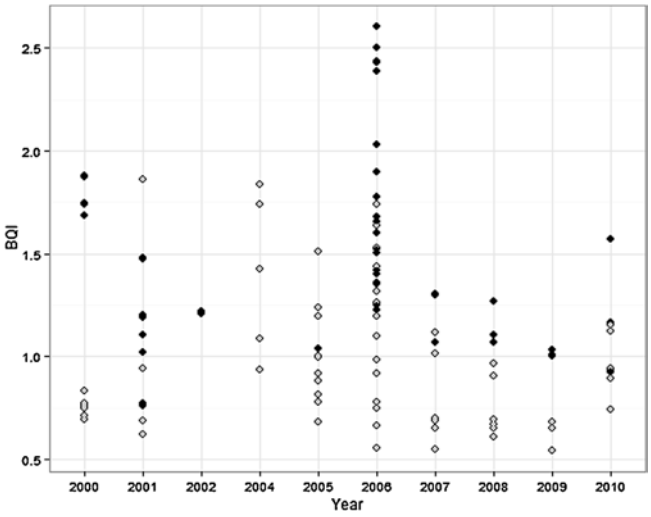

Fig. 2. Temporal variability of BQI values in the analyzed samples. Gray dots represent samples without zebra mussels, black dots – those with zebra mussels.

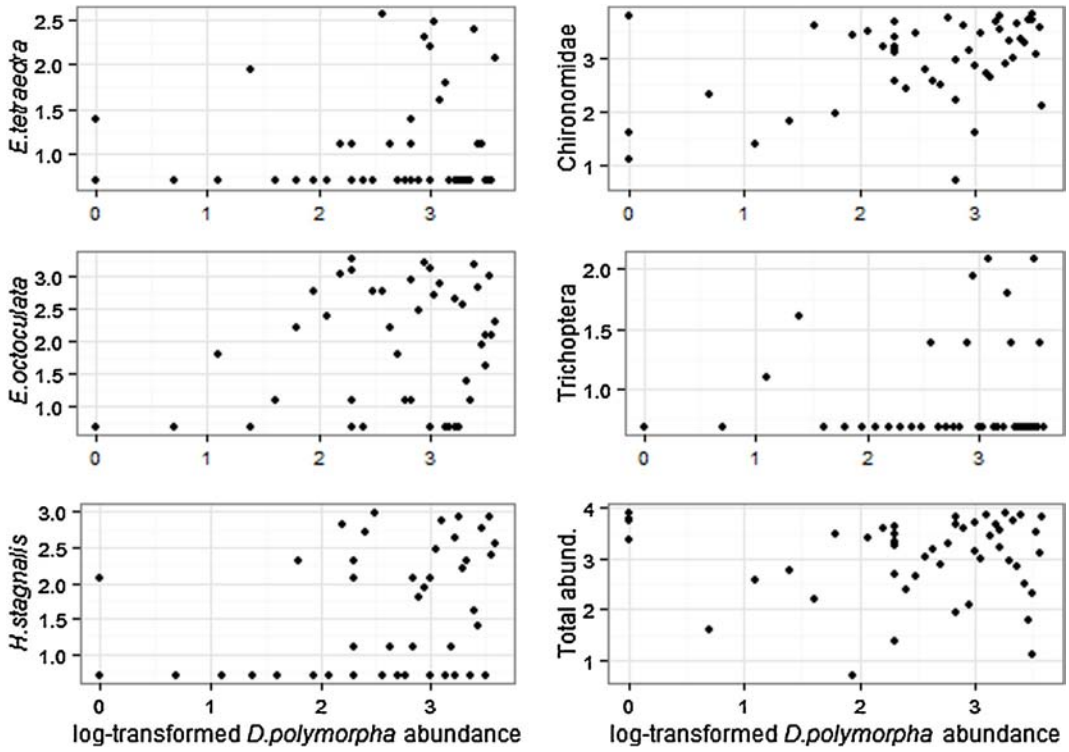

Fig. 3. Abundances (log-transformed) of *E. tetraedra*, *E. octoculata*, *H. stagnalis*, Chironomidae, Trichoptera and total abundance (ind/m^2) versus log-transformed *D. polymorpha* abundance (ind/m^2).

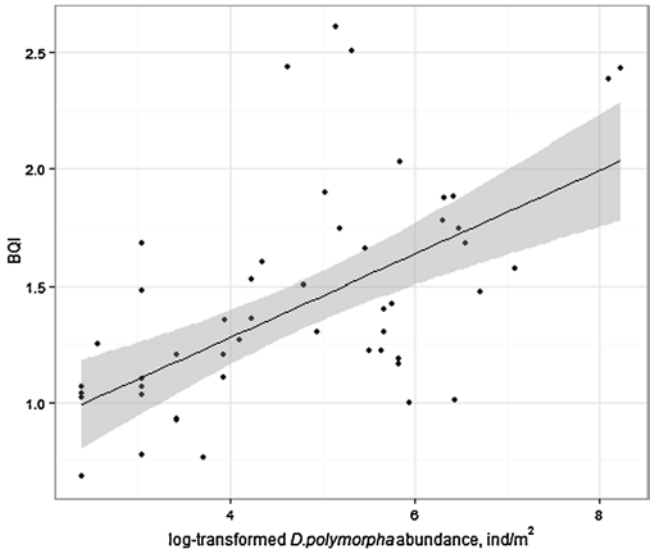

Fig. 4. BQI values in samples with zebra mussels versus *D. polymorpha* abundance (log-transformed) with fitted linear model trendline (R^2 = 33%, r = 0.58, p < 0.001) and standard error represented by shaded area.

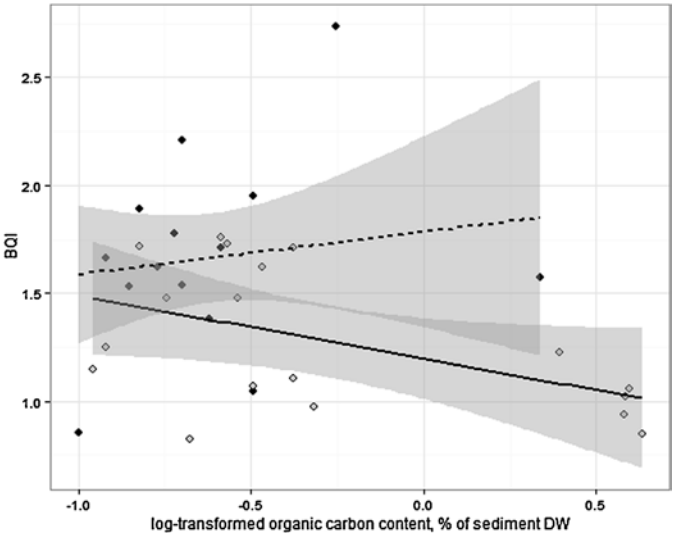

Fig. 5. BQI values calculated for 1999 dataset based on the pre-assigned species sensitivity values (Table 1). Gray dots – samples without zebra mussel: solid regression line (t = 12; p < 0.001; BQI = 1.19 − 0.29 × [log(C_org)]); black dots – samples with zebra mussel: dashed regression line (t = 3; p = 0.006; BQI = 1.89 + 0.36[log(C_org)]).

When verifying the results on 1999 data (applying the pre-assigned sensitivity values), ANCOVA revealed statistically significant effect of zebra mussel presence (F = 5.67; p = 0.02) and marginal effect of organic carbon (F = 3.63, p = 0.07) on the BQI values. Moreover, there was a shift from negative to positive regression in the samples with zebra mussels (Fig. 5).

After applying the dataset corrections, new species sensitivity values and their ranking were obtained (Table 2).

The BQI values calculated with applied data corrections varied within a narrower range (from 0.54 to 2.13) without any significant correlation with zebra mussel abundances (Fig. 6). When the algorithm was tested on 1999 dataset, an insignificant positive trend of BQI values with enhanced organic carbon content still could be detected in the presence of zebra mussel. However impact of organic carbon content remained as the only important factor (F = 6.72, p = 0.01) explaining 21% of variance in BQI data.

4. Discussion

There are at least three scenarios how zebra mussel might compromise the results of benthic quality assessment, if blindly

Table 2
Sensitivity values and re-assigned sensitivity classes for the pre-selected 19 taxa after dataset corrections (1 – very tolerant; 2 – tolerant; 3 – sensitive; 4 – very sensitive). An asterisk denotes species with shifted down sensitivity class comparing to the uncorrected analysis.

Taxa	$ES_{50-0.05}$	Sensitivity class
Oligochaeta	1.4	1
Chironomidae*	1.9	1
*Dreissena polymorpha**	2.0	2
*Glossiphonia complanata**	2.1	2
Gammaridae	2.1	2
Unionidae	2.1	2
Valvata spp.	2.4	2
Hydracarina	2.5	2
*Eiseniella tetraedra**	2.8	2
Ostracoda	2.8	2
*Erpobdella octoculata**	2.9	2
Pisidium spp.	3.0	3
Helobdella stagnalis	3.1	3
Trichoptera	3.1	3
Turbellaria	3.1	3
*Glossiphonia heteroclita**	3.4	3
Sphaerium spp.	3.5	3
Viviparus viviparus	4.1	4
Bithynia spp.	4.7	4

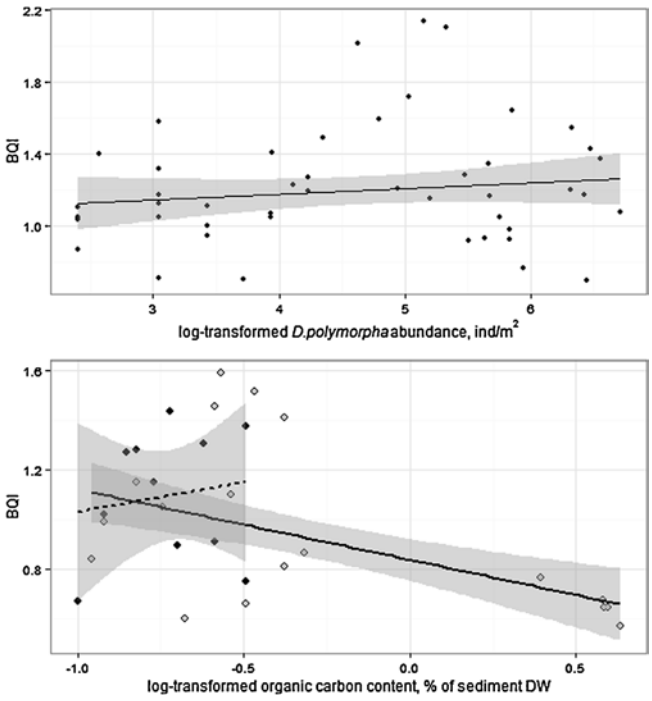

Fig. 6. Top: corrected BQI values in samples with zebra mussels versus zebra mussel abundance (log-transformed) with fitted linear model trendline (R^2 = 2%, r = 0.14, p = 0.32). Bottom: corrected BQI values for 1999 dataset. Gray dots – samples without zebra mussel: solid regression line (t = 11; p < 0.001; BQI = 0.88 – 0.31[log(C_org)]); black dots – samples with zebra mussel: dashed regression line (t = 1; p = 0.35; BQI = 0.39 + 0.24[log(C_org)]).

incorporated into the data analysis: (I) by altering species richness; (II) by altering species abundance; (III) by restructuring the whole community in sites highly modified by zebra mussel colonies. Particularly, in the case with BQI calculation (see Eq. (1)), all of its components can be potentially affected: number of observed species, abundance of a species, total abundance, ES_{50} and $ES_{50-0.05}$ values.

As it was reported from earlier studies, many benthic invertebrates tend to aggregate in habitats modified by zebra mussels (Karatayev et al., 2002; Reed et al., 2004; Zaiko et al., 2009). In soft-bottom environments, zebra mussels provide substrata and shelter for the epifaunal (e.g. *A. aquaticus*, *C. macrura*, *E. octoculata*, *H. stagnalis*, *R. auricularia*) and infaunal invertebrates (e.g. Chironomidae, Ceratopogonidae, *E. tetraedra*) (Bially and MacIsaac, 2000; Minchin and Zaiko, 2013). Both detritovorous and carnivorous species benefit from the structural complexity and resources availability (produced biodeposits, sheltered prey items) enhanced by zebra mussel colonies (Zaiko et al., 2009). This explains the fact that a few taxa in our study have demonstrated high level of association with zebra mussel presence and/or abundance.

Except for species significantly correlating with zebra mussel abundance (Fig. 3), there were at least 7 taxa observed exclusively in the presence of zebra mussel. Consequently, the enhanced species richness, total abundances and ES_{50} values might be affected by zebra mussel presence as well.

On the other hand, there were three species (*G. aquaticus*, *G. concolor* and *P. antipodarum*) observed exclusively devoid of zebra mussels. These species were reported from the west-coast monitoring stations with the prevalence of fine silty mud in sediments (Trimonis et al., 2003), where conditions were highly unfavorable for the zebra mussel population establishment (Fenske et al., 2013). However due to their low abundances and occurrence below 5%, these three species had minor effect on the estimated BQI values.

Based on the species ranking according to their sensitivity (original dataset), *D. polymorpha* hit the third quartile suggesting it as a rather sensitive species. However its high tolerance to variable environmental conditions and different levels of anthropogenic pressure is known from multiple observational and experimental studies worldwide (Claudi and Mackie, 1993; Shkorbatov et al., 1994; Fenske et al., 2013). Therefore, we suspect that sensitivity values determined for some other species in this study could be also an artifact of their association with presence or high abundance of zebra mussels. Although for the part of the considered species (e.g. Oligochaeta, Chironomidae, *Bithinia*, *Erpobdella*, *Glossiphonia*, *Sphaerium*) sensitivity scores were consistent with the results reported by other studies in the region (Osowiecki et al., 2008; Kotta et al., 2012; HELCOM, 2013), others could be artificially elevated or vice versa demoted due to their preference for modified habitats or other particular inter-specific relationships with zebra mussel.

Referring to the results presented, the simplest solution is to eliminate samples with zebra mussel (or any other IAS with strong impact on habitats and communities) from the benthic quality assessment. However, in many invaded ecosystems this would imply exclusion of significant part of the data from the analysis. For instance, in the analyzed dataset from the Curonian Lagoon samples with zebra mussels comprised nearly 50% of the monitoring data and were obtained from 13 locations (out of 15 sampled). Exclusion of these samples would significantly reduce the representativeness of the assessment and robustness of the conclusions.

On the other hand, due to the patchy and non-persistent distribution of zebra mussels (Olenin and Daunys, 2004; Zaiko et al., 2009, 2014), it is difficult to estimate precisely the probability of finding the species in a particular location. It means that *a posteriory* exclusion of samples from the analysis should be applied, thus affecting the overall cost-effectiveness of the monitoring program. Therefore in this study we have demonstrated a framework of the dataset correction that proved to be effective enough minimizing the zebra mussel effect on the BQI assessment outcome.

The applied corrections have resulted in a rather logical shift in sensitivity class of 6 species (Table 2). This time zebra mussel pooled within the tolerant species group that matched our general expectations based on expert knowledge. Five other species (Chironomidae, *Glossiphonia complanata*, *G.heteroclita*, *E. tetraedra* and *E. octoculata*) have been also assigned lower sensitivity class compared to the uncorrected data analysis.

The BQI values calculated on the corrected dataset have demonstrated better responsiveness to the considered pressure (eutrophication, expressed by the organic carbon content) with minimized undesired "noise" caused by the presence of invasive ecosystem engineer (Fig. 6). Thus the reliability and overall robustness of the environmental status assessment was improved.

Although data correction framework presented here showed good results in our BQI calculation exercise, the environmental context and ecosystem peculiarities should be considered before applying this approach. Our experience could be applied for other coastal ecosystems invaded by the zebra mussel or any other IAS with similar bioinvasion impacts, after a proper validation and ecosystem-specific adjustments (e.g. for sensitivity values and correlations).

5. Conclusions

The results of the BQI assessment exercise presented here demonstrate that several important characteristics of the indicator (including its responsiveness, sensitiveness, predictability, accuracy) could be compromised due to the impact of IAS present in an ecosystem. Zebra mussel ability of modifying benthic habitats and forming local patches of elevated biological diversity may bias the results of benthic quality assessment by showing false improvement of ecological status. If not considered in the course of the assessment, any species richness-based index may reflect IAS impact rather than anthropogenic pressure effect. Proper adjustments of ecological status assessment are desirable for the ecosystems strongly affected by IAS.

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Appendix 13.

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Application of signal detection theory approach for setting thresholds in benthic quality assessments

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ABSTRACT

The European Marine Strategy Framework Directive requires EU Member States to prepare national strategies and manage their seas to achieve good environmental status (GES) by 2020. There are many multimetric indices proposed as indicators of the ecological quality of the benthic environment. Their functionality and utility are extensively discussed in the literature. Different frameworks are suggested for comparative assessments of indicators with no agreement on a standardized way of selecting the most appropriate one. In the current study, we apply signal detection theory (SDT) to evaluate the specificity and sensitivity of the Benthic Quality Index (BQI), its response to the eutrophication pressure, and its performance under the effects of estuarine water outflow. The BQI showed acceptable response to total nitrogen, total phosphorus and chlorophyll-*a* concentrations in the study area. Based on the results, we suggest using SDT for setting GES thresholds in a standardized way. This aids a robust assessment of the environmental status and supports differentiation between the quality classes.

1. Introduction

The European Marine Strategy Framework Directive (MSFD) requires EU Member States to align national legislative policies and appropriately manage their seas in order to achieve good environmental status (GES) by 2020 (MSDF; European Commission 2008/56/EC). GES is defined as 'clean, healthy and productive seas within their intrinsic conditions, and the sustainable use of the marine environment'. The directive requires application of a set of indicators for environmental status assessment. When GES criteria are not met, the corresponding measures for achieving them must be specified and undertaken.

Obviously, an adequate and efficient management strategy for the improvement of environmental status implies a robust and reliable status assessment. The crucial step here is the selection of appropriate indicators, therefore many research projects specifically address this issue (Ferreira et al., 2011; Rice et al., 2012; ICES, 2013). A few selection criteria have been suggested, including (but not limited to) scientific basis, responsiveness, range of applicability, data availability, practicality, harmonization, accuracy and confidence (Rice and Rochet, 2005; Niemeijer and de Groot, 2008; Elliott, 2011). Several evaluation methods and conceptual frameworks have been discussed to facilitate decision-making (Borja and Dauer, 2008; Kershner et al., 2011; ICES, 2013). The responsiveness of an indicator is often distinguished among the selection criteria (Rombouts et al., 2013). Once an indicator has been developed, its performance in terms of sensitivity (response to an existing disturbance), specificity (resistance to the noise or non-targeted disturbances) and the accuracy in relation to the actual response can be evaluated (Murtaugh, 1996).

It is assumed that benthic species and communities reflect natural and anthropogenic changes in marine ecosystems as they are unable to avoid unfavourable conditions, have a long reproductive cycle, accumulate changes over time and occur at various depths (Zettler et al., 2007). A series of multimetric indices have been proposed to supply synoptic information about the state and ecological quality of the benthic environment, e.g. the Benthic Quality Index (BQI; Rosenberg et al., 2004; Leonardsson et al., 2009), the AZTI Marine Biotic Index (AMBI; Borja et al., 2000), the Biotic Index (BENTIX; Simboura and Zenetos, 2002), the Benthic Opportunistic Polychaeta Amphipoda Index (BOPA; Dauvin and Ruellet, 2007) and the Benthic Opportunistic Annelida Amphipods Index (BO2A;

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Dauvin and Ruellet, 2009). Yet the performance of these indicators is unlikely to be consistent across habitats and ecosystems, since bottom-dwelling organisms are not equally sensitive to different types of anthropogenic and natural disturbances (Buhl-Mortensen et al., 2009), or environmental conditions (Tagliapietra et al., 2009). Many authors agree that eutrophication, chemical pollution and mechanical disturbance of the sea bottom are the major anthropogenic pressures determining changes in macrofauna abundance, distribution and species composition (McQuatters-Gollop et al., 2009; Van Hoey et al., 2010; Rice et al., 2012). Among those, eutrophication is often emphasized as a particularly large-scale driving force of ecosystem changes, having multiple indirect effects and therefore not being easily quantifiable by direct measurements (Van Hoey et al., 2010). Therefore, detection of eutrophication effects relies mostly on the sensitivity of selected indirect measurements and synoptic indicators (such as benthic indices).

Many studies have aimed to test and validate benthic indicators, applying different analytical frameworks and statistical approaches. For instance, the responsiveness of the BENTIX index (Simboura and Zenetos, 2002) to water quality parameters (dissolved oxygen, particulate and total organic carbon) was assessed using linear regression. Factorial analysis was used by Muxika et al. (2007) when validating benthic quality assessment performed with the AMBI Index (Borja et al., 2000). Diaz et al. (2004) assessed the functionality of 64 benthos-related indices applying qualitative comparison based on a comprehensive literature review.

Among different frameworks suggested for quality analysis of GES indicators, there is still little agreement on a uniform approach for a robust and standardized selection of appropriate metrics (Mazik et al., 2010; HELCOM, 2012). Here, we demonstrate the application of signal detection theory (SDT) to identify and quantify the indicator response to a particular anthropogenic pressure. This method has been extensively used in medical studies, but has also been considered for ecological application (Murtaugh, 1996; Hale and Heltshe, 2008). In the current study, we assess the specificity and sensitivity of the Benthic Quality Index (BQI), its response to the eutrophication pressure, and its performance in relation to the soft-bottom habitats affected by estuarine water outflow.

2. Materials and methods

2.1. Study area

The performance of the BQI was assessed in relation to the soft-bottom habitats in the Lithuanian coastal zone, south-eastern Baltic Sea (Fig. 1). Due to high wave exposure there is no oxygen deficiency in the near-bottom layer. Salinity in the study area varied from 6.3 to 7.4‰ outside the plume and decreased down to 3.3‰ in the areas exposed to a freshwater outflow from the Curonian Lagoon (the plume zone). Approximately 60 different benthic macrofauna species have been reported in this area (Olenin et al., 1996). Hard-bottom communities are dominated by the blue mussel *Mytilus edulis* and the barnacle *Amphibalanus improvisus*, whereas sandy bottoms are dominated by the spionid polychaetes *Pygospio elegans* and *Marenzelleria* sp. or the bivalve *Macoma baltica* (Bubinas and Vaitonis, 2003; Olenin and Daunys, 2004). Eutrophication is considered to be one of the main pressures affecting water quality in the study area (Olenin and Daunys, 2004).

2.2. Data collection

A long-term (May–September samplings between 1984 and 2012) benthic macrofauna data set covering six monitoring sites (Fig. 1) was used for assigning the species sensitivity values ($ES_{50\text{-}0.05}$), as described by Leonardsson et al. (2009). For the BQI calculation and responsiveness analysis, data (2005–2011) on macrofauna diversity and abundance (ind/m^2), and summer averages (June–August) of total phosphorus (TP mg/l), total nitrogen (TN mg/l) and chlorophyll-*a* (chl-*a* μg/l) concentrations were used. These parameters were chosen as "direct measures" of eutrophication, suggested among others within the MSFD (Ferreira et al., 2011).

Benthic samples were collected from the soft-bottom habitats at depths ranging from 13–20 m, sieved on-site through a 0.5 mm mesh and processed according to the standard HELCOM recommendations (COMBINE manual). Data on TP and TN were collected as part of the national monitoring programme (unpublished data, Environment Protection Agency), and chl-*a* data were retrieved from the MEdium Resolution Imaging Spectrometer (MERIS), the ENVISAT satellite of the European Space Agency.

The final data set used for the analysis consisted of 77 samples collected from six locations (Fig. 1) within the coastal zone.

2.3. BQI index calculations

When testing the responsiveness of the BQI to the eutrophication pressure (expressed by TP, TN and chl-*a* concentrations), a one-year lag was applied for the index values in respect of pelagic parameters. Instant effects (no lag) were less likely in our study due to the timing of pelagic and benthic samplings (June–August and May–September respectively). One-year lag was also supported by the best statistical response using multiple linear regression ($r = 0.30$, $p = 0.08$) of the BQI to environmental variables compared to no or two-year lag applications ($r = 0.06$, $p = 0.80$ and $r = 0.04$, $p = 0.86$ respectively).

Since the original version of the BQI (Rosenberg et al., 2004) is known to be sampling effort dependent (Fleischer et al., 2007), the adjusted calculation was applied (Fleischer and Zettler, 2009)

$$\mathrm{BQI} = \left(\sum_{i=1}^{n}\left(\frac{A_i}{A_{tot}} \times ES_{50_{0.05i}}\right)\right) \times \log(ES_{50}+1) \times \left(1 - \frac{5}{5+A_{tot}}\right) \quad (1)$$

In the above equation, n denotes the observed species number. A_i stands for the abundance of the species i (ind m^{-2}) and A_{tot} is the sum of all individuals (ind m^{-2}). Finally, $ES50_{0.05i}$ is the sensitivity/tolerance value for the species i and ES_{50} denotes the estimated species number among 50 randomly picked individuals within a square metre (Hurlbert Index). The sensitivity value of a species was set to the 5th percentile of the ES_{50} ($ES50_{0.05i}$) in the samples where the species was present.

2.4. Signal detection theory

According to SDT, the sensitivity and specificity of an indicator can be calculated according to four possible outcomes – hits (correct interpretation of a true response – true positives), misses (inability to detect a true response – false negatives), false alarms (false detection of a response – false positives) and correct rejections (correctly interpreted missing response – true negatives) – given that the target condition ("gold standard") is known. Receiver operating characteristic (ROC) curves provide a visual tool for assessing the accuracy of an indicator, by plotting the probability of the true positives (sensitivity) against the probability of the true negatives (specificity). The area under the ROC curve (AUC) can be used as a measure of the indicator response. A perfect indicator should have an AUC of 1, whereas 0.5 is a measure of a non-informative indicator (Murtaugh, 1996). In ecological studies, AUC values ≥0.8 are considered to indicate an excellent and ≥0.7 an acceptable response (Hale and Heltshe, 2008).

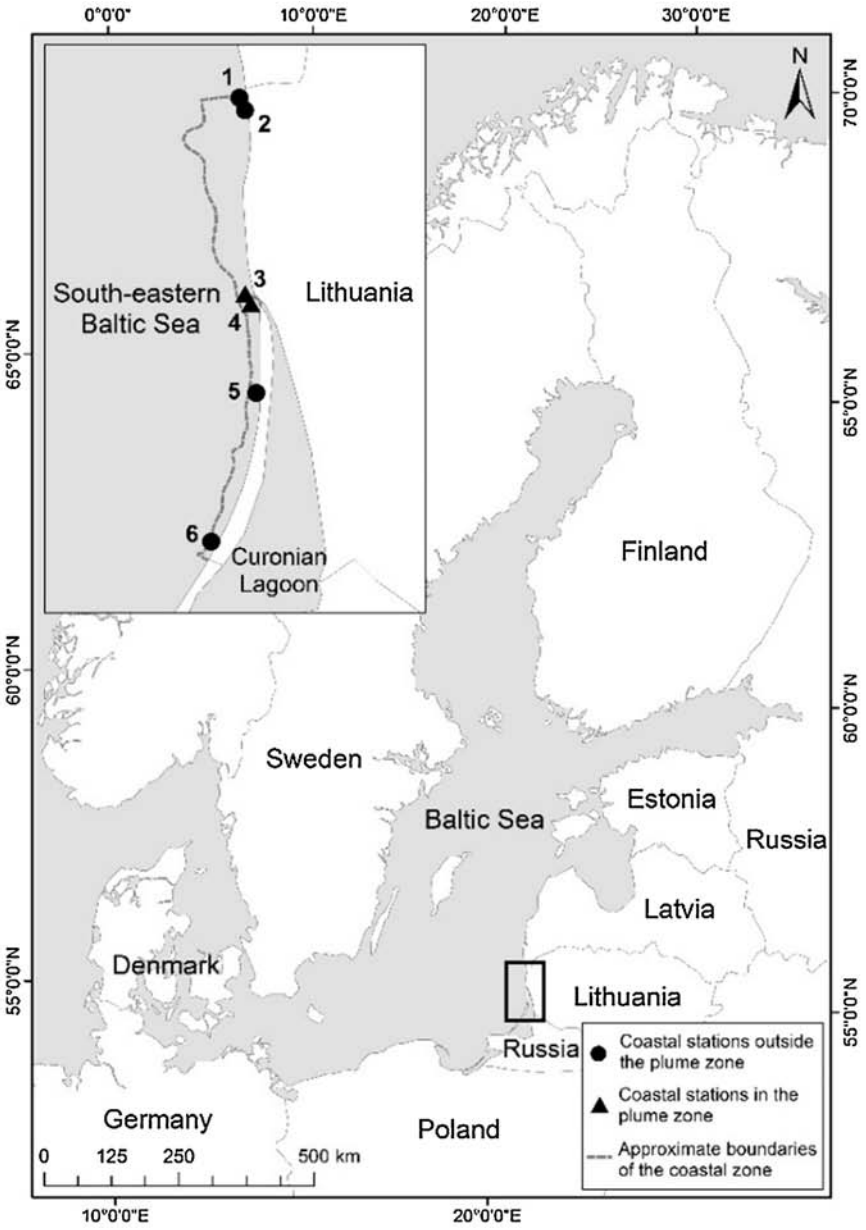

Fig. 1. Study area and sampling sites in the south-eastern part of the Baltic Sea. Black triangles denote sampling stations exposed to the reduced salinity conditions due to the freshwater outflow from the Curonian Lagoon (the plume zone); black dots – sampling stations outside the plume zone. Dashed lines indicate the approximate boundaries of the coastal zone and correspond to the 20 m isobaths.

The predictive ability of the indicator is described by the positive predictive value (PPV: the probability of the true positives) and the negative predictive value (NPV: the probability of the true negatives) (Murtaugh, 1996). The PPV and NPV values vary, according to the prevalence of the target values in the analysed parameter (values at or above the good water quality threshold, as defined for this study). For example, at low prevalence of the target values a correct (true positive) response will only be attained with an accurate indicator, implying a low rate of false positives (Swets et al., 2000). Thus, by using PPV and NPV, the probability of getting a correct response can be evaluated against the risks of making wrong decisions. This approach can be used to set indicator thresholds for distinguishing impacted sites from undisturbed ones.

To test and verify the response of the BQI using ROC curves, the calculated values were related to gold standards based on TP, TN and chl-*a* concentrations. Since the quality class threshold between "good" and "moderate" status, *sensu* the Water Framework Directive (WFD; European Commission 2000/60/EC), is critical for distinguishing between substantial deviation and the natural range of the indicator values, it was applied for setting the gold standard (or target) values in the current SDT analysis (Table 1).

Table 1
Good/moderate status thresholds defined for the Lithuanian coastal area (WFD Lithuania Surface water bodies methodological material, 2009) and applied in the current STD analysis.

Eutrophication parameter	Outside the plume zone	Within the plume zone
Chl-*a* concentration (μg/l)	≤4.8	≤25.7[a]
TP concentration (mg/l)	≤0.026	≤0.026[b]
TN concentration (mg g/l)	≤0.25	≤0.25[b]

[a] Salinities <4‰.
[b] Salinities >4‰.

2.5. Statistical analyses

Linear regression was applied to relate calculated BQI values to the environmental parameters (chl-*a*, TP, TN concentrations). The Bonferroni α-correction for α was applied for multiple pairwise tests. The Primer software package (Clarke and Warwick, 2001) was used for calculation of the Hurlbert Index (ES_{50}). The graphical visualizations (including ROC curves), threshold estimations and analyses were performed in the R v3 statistical computing environment (R-project, 2014).

3. Results

The calculated BQI values ranged from 1.7 to 3.4 with no apparent difference between plume area and the rest of the coastal zone. The average chl-*a* and TN concentrations were significantly higher within the plume zone than outside the plume zone (chl-*a*: 27.5 ± 1.1 μg/l and 4.1 ± 0.3 μg/l respectively, $t = -25.018$, $p = 0.0001$; TN: 0.51 ± 0.04 mg/l and 0.35 ± 0.02 mg/l respectively, $t = -3.783$, $p = 0.0006$). This is supported by a significant negative relationship between salinity and TN concentrations in the plume ($r = -0.72$, $p < 0.001$, salinity range between 3.3 and 7.1‰), while outside the plume this relationship was negligible ($r = -0.02$, $p < 0.001$, salinity range between 6.3 and 7.4‰). TP concentrations were similar within and outside the plume with no significant differences between average values (0.038 ± 0.002 mg/l and 0.034 ± 0.003 mg/l respectively) and a very weak negative relationship with salinity.

For BQI validation, its relationships with chl-*a*, TN and TP summer concentrations were first analysed applying linear regression. When partitioning the plume effect (relating the BQI values to eutrophication parameters separately for sampling sites within and outside the plume area), a statistically significant negative correlation with chl-*a* concentrations ($r = -0.27$; $p = 0.02$) was revealed outside the plume zone only, while no significant relationships were detected for TN and TP either within or outside the plume zone. In general, the analysed eutrophication parameters had higher variability within the plume area (chl-*a* concentration range was between 14.7 and 41.3 μg/l, and TN and TP concentration ranges were 0.25–0.87 mg/l and 0.021–0.057 mg/l respectively) compared with their values outside the plume zone (chl-*a* concentration range was 1.7–10.5 μg/l, and TN and TP concentration ranges were 0.15–0.51 mg/l and 0.016–0.065 mg/l respectively) (Fig. 2).

The SDT analysis was performed separately for the studied coastal areas. In accordance with the AUC classification by Hale and Heltshe (2008), an acceptable BQI response (AUC = >0.70) was revealed to all analysed eutrophication parameters measured outside the plume zone. Within the plume zone, however, the index response to chl-*a* and TP concentrations was qualified as poor, but excellent for TN concentration (Fig. 3).

The relation between the BQI values and the chl-*a* concentrations outside the plume zone was used for setting the threshold between "acceptable" and "unacceptable" status of water quality (Fig. 4). BQI thresholds were assigned at different specificity and sensitivity levels.

The most accurate BQI threshold, according to the sum of sensitivity and specificity values estimated from the index response to chl-*a* (i.e. ROC curves), was 2.56 (specificity and sensitivity 0.75 and 0.86 respectively; Fig. 4). In our data set, the prevalence of the chl-*a* values falling within the target range (between the good and moderate water quality classes) was 0.69 (16 out of 23 samples). At this prevalence, the most accurate BQI response showed an ability to correctly identify "acceptable" conditions in 89% of cases (positive predictive value, PPV) and "unacceptable" conditions in 68% of cases (negative predictive value, NPV) (Fig. 4, Table 2).

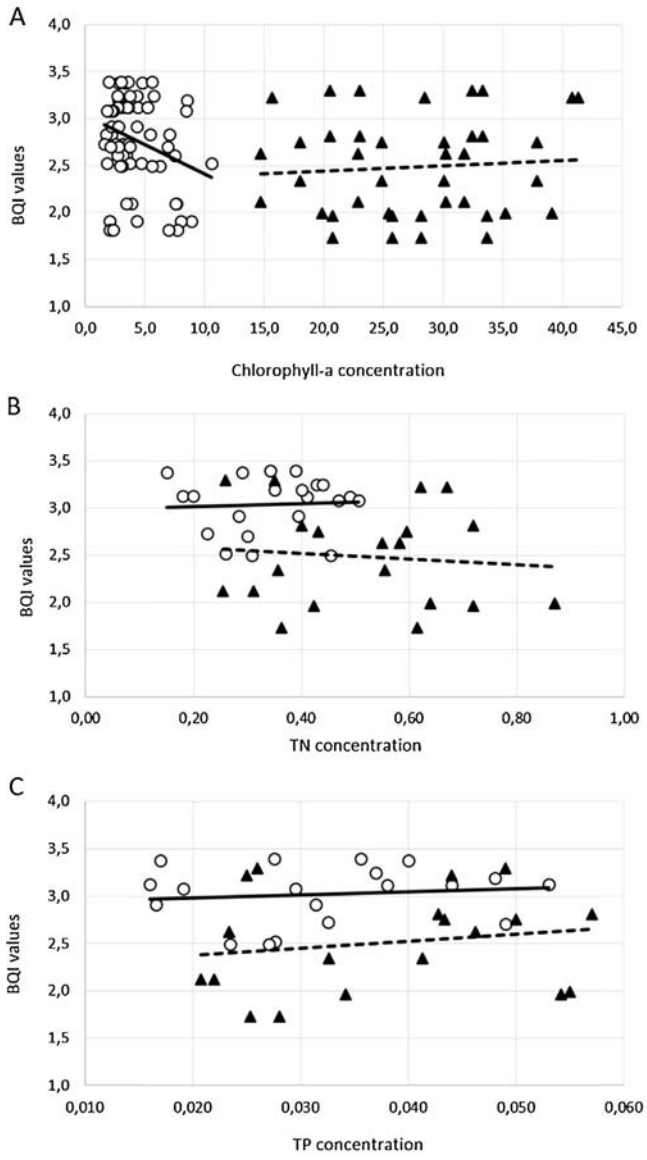

Fig. 2. BQI values versus chl-*a* concentrations (A), TN (B) and TP (C) with fitted linear model trend lines for the samples taken outside the plume (white dots, solid line) and within the plume zone (black triangles, dotted line).

In a healthy environment, an assessment should rely more on NPV rather than on PPV and vice versa. For instance, when applying a strict threshold at 2.45 (BQI boundary between the good and moderate environmental status classes), the PPV is higher (90%) and NPV is lower (55%) compared to a threshold set at the most accurate BQI response (2.56). A lenient threshold at 3.05 results in a comparatively low PPV and high NPV (80% and 100% respectively) (Table 2).

4. Discussion

The BQI is one of the most widely used multimetric indices for macrofauna status assessment (Rosenberg et al., 2004; Fleischer and Zettler, 2009; Leonardsson et al., 2009). Although designed for application in marine areas, it is also considered to be suitable for different environments provided that the assigned species' tolerance/sensitivity values are based on individual data sets and

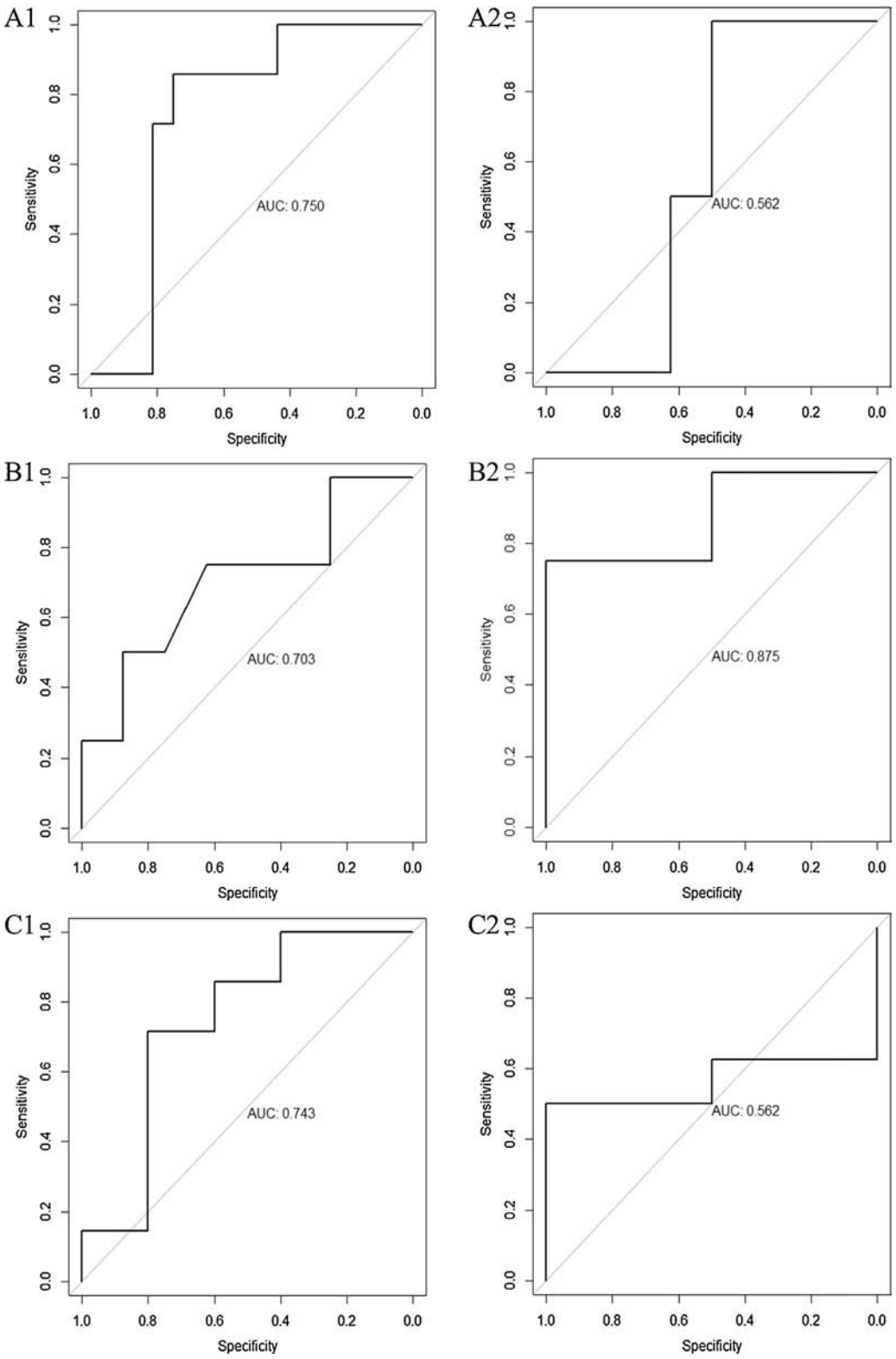

Fig. 3. ROC (receiver operating characteristic) curves for BQI response to chl-*a* (A), TN (B) and TP (C) concentrations in the study area outside the plume zone (left column) and within the plume zone (right column).

are site-specific (Zettler et al., 2007). The index is assumed to be ecosystem relevant and reproducible since it has been tested and validated in different marine ecosystems with varying environmental conditions, however its performance can be affected by the salinity gradient and the presence of invasive species (Labrune et al., 2006; Zettler et al., 2007; Zaiko and Daunys, 2015).

Our results revealed an acceptable BQI response to the analysed eutrophication parameters for coastal waters. These results support the applicability of the BQI for benthic quality assessment in relation to nutrient/organic pollution (eutrophication pressure) in the exposed coastal areas of the brackish Baltic Sea. However, the response was not detected with the traditional statistical approach

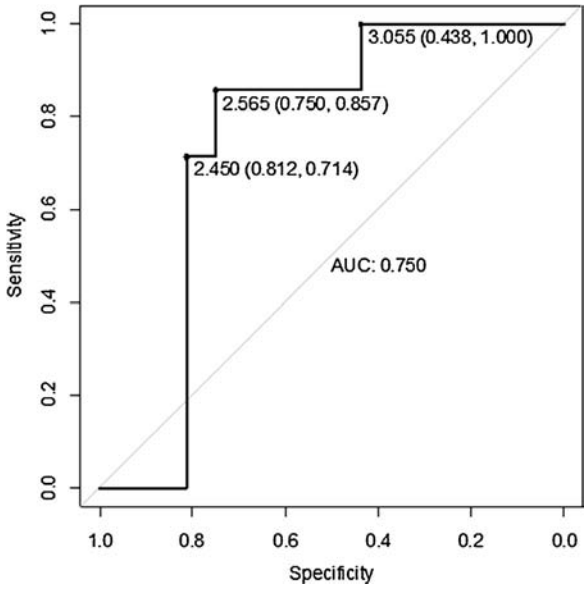

Fig. 4. ROC (receiver operating characteristic) curves for the BQI, as a response to the chl-*a* concentration. The steps denote proposed threshold values (strict – 2.45, the most accurate – 2.56 and lenient – 3.05). Numbers in brackets indicate specificity and sensitivity values respectively.

(linear regression). The effect of organic pollution on benthic communities is unlikely to be straightforward and therefore difficult to measure. When testing an indicator's responsiveness, ideally the assessment should be performed along the gradient of the selected pressure (i.e. eutrophication in our case), excluding any untargeted disturbances (noise effects). This is, however, an unlikely case when working with typical field data and particularly with those from coastal areas, where multiple natural and anthropogenic factors are often simultaneously present.

In the Baltic Sea, eutrophication constitutes one of the most important pressures affecting different ecosystem components – from phytoplankton to the benthic communities (HELCOM, 2009). Many parameters have been proposed for measuring the eutrophication effect, e.g. optical water column properties, oxygen concentration, frequency of algae blooms, chlorophyll-*a* and nutrient concentrations. Only a few of them could be used for the pressure-response analysis due to the lack of consistent long-term observations. An increase in nutrient concentrations directly affects phytoplankton development and chl-*a* concentration. During succession, the phytoplankton biomass turns to organic material and becomes a food supply for the benthic communities in the case of transfer of material to the near-bottom layer or sediment as supported by vertical flux. Previous studies demonstrate that relationships between macrozoobenthos biomass and nutrients (TN and TP concentrations) were little affected by coastal exposure, and benthic invertebrates were more sensitive to changing TN concentrations in the shallower areas than in the deeper ones (Kotta et al., 2007). The indirect eutrophication effects such as altered species diversity or the proportion of tolerant and sensitive species do not assert instantly and may accumulate over time. They may also emerge later depending on the intensity of the impact and involved mechanisms (changed reproduction rate, modified feeding activity, increased physiological stress, etc.) (Kotta et al., 2007; Grall and Chauvaud, 2002; Heip, 1995). As a result, a certain lag period is typically involved in relationships between benthic and pelagic parameters (e.g. Snickars et al., 2014).

Signal detection theory provides an appropriate approach for determining the underlying response. As a non-parametric method it is insensitive to general statistical assumptions, but nonetheless is able to provide estimates of indicator sensitivity and specificity as well as its predictive value. This method proved to be effective in uncovering the BQI response to eutrophication parameters in our case study. However, we found some inconsistencies in the BQI response to eutrophication parameters assessed by SDT in two cases (i.e. poor response to chl-*a* and TP concentrations within the plume zone). Stronger BQI response to TN concentrations compared to TP driven by organic matter production is more likely in the plumes, since highly eutrophied areas with a low-salinity regime are known to be predominantly N-limited (Tamminen and Andersen, 2007). On the other hand, effects of a strong spatial and temporal salinity and organic enrichment gradients in the plume zone may not necessarily coincide. Low salinity has been reported as the driving force of the macrofauna distribution in many estuarine-like ecosystems (e.g. Boesch, 1977; Ysebaert et al., 1993; Bonsdorff, 2006), where its effect was asserting independently from the influence of organic enrichment (mud content, chl-*a* concentrations) (e.g. Ysebaert and Herman, 2002). It is obvious, that even being of the same resistance to organic enrichment, different species may have individual salinity tolerances, particularly in the plume areas with transition between freshwater and critical salinity range of 5–8‰. For instance, genuine brackish species (e.g. *Marenzelleria* sp. or *Hediste diversicolor*) respond to low salinity differently compared to e.g. *Macoma balthica* (Fritzsche and Oertzen, 1995). Consequently, the integrated measures such as BQI or other univariate indices may have limited capacity to reflect such highly variable responses of multiple species, hence resulting in weak relationships with abiotic parameters in the complex environments.

The overarching purpose of any environmental indicator is to distinguish between healthy and degraded environments and provide a scientifically based reasoning for undertaking appropriate measures to improve the ecological status. The application of the SDT approach can assist in assessing the performance of candidate environmental metrics under particular conditions, setting the threshold values and evaluating water quality status in a robust and scientifically sound way. The most accurate index threshold suggested by SDT might not always be the preferred choice, as management effort may be advisable in some cases, when degradation is less pronounced and natural recovery is still feasible. On the other hand, an environmental manager assessing the status of particularly valuable or protected areas might prefer the lower risk of overlooking deterioration and therefore will need to maximize NPV values and set a lenient threshold for the index. If the BQI is assessed in a largely affected area, the positive predictions will be more accurate than the negative ones, hence maximized PPV values and a stricter threshold for the index are advisable. Considering these aspects would help to support the adequate management effort

Table 2
Suggested BQI thresholds based on the response to chl-*a* concentrations outside the plume zone, with corresponding estimates of prevalence, specificity, sensitivity, PPV and NPV (based on SDT approach).

BQI thresholds for chl-*a* concentration values	Prevalence of the target chl-*a* values (between good and moderate conditions)	Specificity	Sensitivity	PPV (%)	NPV (%)
2.45 – strict	0.69	0.81	0.71	90	55
2.56 – the most accurate	0.69	0.75	0.86	89	68
3.05 – lenient	0.69	0.44	1.00	80	100

and appropriate remediation measures on site (Hale and Heltshe, 2008).

SDT provides a practical tool to validate indicator thresholds and select good environment status (GES) boundaries for a particular area. Based on the SDT analysis results, one could decide whether an indicator is representative enough for detecting the particular pressure. Depending on the targets set, information retrieved from the SDT analysis can be used for designing the monitoring programme and answering practical ecological and management questions, e.g. how dense the sampling network should be to detect the pressure and assess the environmental status in light of the specific conditions, potential noise factors and uncertainties involved.

5. Conclusions

Although the traditional data exploration methods showed a weak or no relation between the BQI and the selected eutrophication parameters, SDT indicated a clear BQI response to the eutrophication pressure in the studied area. The response was affected by the freshwater outflow from the Curonian Lagoon, though. Signal detection theory (ROC curves, PPV and NPV approach) can be proposed as a standardized method to assess the responsiveness of an indicator to a particular pressure and set appropriate threshold values for the environmental status assessment. The thresholds, however, should be adjusted for a particular area or ecosystem to fit the environmental and management context.

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Spatial prediction of demersal fish diversity in the Baltic Sea: comparison of machine learning and regression-based techniques

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Marine spatial planning (MSP) is considered a valuable tool in the ecosystem-based management of marine areas. Predictive modelling may be applied in the MSP framework to obtain spatially explicit information about biodiversity patterns. The growing number of statistical approaches used for this purpose implies the urgent need for comparisons between different predictive techniques. In this study, we evaluated the performance of selected machine learning and regression-based methods that were applied for modelling fish community indices. We hypothesized that habitat features can influence fish assemblage and investigated the effect of environmental gradients on demersal fish diversity (species richness and Shannon–Weaver Index). We used fish data from the Baltic International Trawl Surveys (2001–2014) and maps of six potential predictors: bottom salinity, depth, seabed slope, growth season bottom temperature, seabed sediments and annual mean bottom current velocity. We compared the performance of six alternative modelling approaches: generalized linear models, generalized additive models, multivariate adaptive regression splines, support vector machines, boosted regression trees and random forests. We applied repeated 10-fold cross-validation, using accuracy as the measure of model quality. Finally, we selected random forest as the best performing algorithm and implemented it for the spatial prediction of fish diversity from the Baltic Proper to the Kattegat. To obtain information on the data reliability and confidence of the developed models, which are essential for MSP, we estimated the uncertainty of predictions with standard deviation of predictions obtained from all the trees in the ensemble random forest method. We showed how state-of-the-art predictive techniques, based on easily available data and simple Geographic Information System tools, can be used to obtain reliable spatial information about fish diversity. Our comparative work highlighted the potential of machine learning method to reduce prediction error in modelling of demersal fish diversity in the framework of MSP.

Keywords: Baltic Sea, demersal fish diversity, ecological modelling, ecosystem-based management, machine learning, marine spatial planning, random forest.

Introduction

Marine spatial planning (MSP) is considered a valuable tool in ecosystem-based management of marine areas (Caldow *et al.*, 2015). Different biological, physical, and socioeconomic criteria can be combined in this management approach (Young *et al.*, 2007). In the pursuit of ecological goals, MSP has the potential to minimize the disadvantages of single-species management and to reduce problems arising from discrepancies between ecological and jurisdictional boundaries (Crowder and Norse, 2008). Despite the benefits of MSP, management authorities encounter considerable technical challenges due to limited and inconsistent information about the environment (Foley *et al.*, 2010). Spatially continuous environmental data, such as the full-coverage maps, are required for proper decision-making. During the last decade, much effort was devoted to collecting data from a wide range of scientific fields, and many organizations have been involved in common databases (Caldow *et al.*, 2015). These joint efforts have provided valuable possibilities for the large-scale mapping of

biological communities and habitats, which can be helpful for decision-makers at least during the initial phase of management plan development (Joy and Death, 2004).

In recent years an increasing amount of studies presenting an integrative approach to biodiversity conservation has been observed (Stuart-Smith *et al.*, 2013; D'Agata *et al.*, 2014). In this approach, different aspects of diversity within species assemblages can be quantified (taxonomic, phylogenetic and functional diversity). However, taxonomic type of diversity, expressed, e.g. by species richness, is the most commonly used component of diversity and may be considered as a surrogate for other components (Devictor *et al.*, 2010). The role of ecologists is to provide, using their best scientific knowledge, quantitative and spatially explicit information about biodiversity patterns (Pittman *et al.*, 2007). However, even in intensively studied areas, the spatial distribution of species cannot be fully monitored due to technical limitations and economic reasons (Leathwick *et al.*, 2006a). A reliable and cost-effective technique used to fill in these inevitable gaps in the biological information is predictive modelling (Guisan and Zimmermann, 2000; Elith *et al.*, 2006). The aim of this approach is firstly to relate by the model the biological survey data from sampled sites to environmental predictors and secondly to provide a map of investigated measure across whole region of interest (covering also unsampled areas) based on prediction of developed model (Ferrier and Guisan, 2006).

Predictive modelling has been widely used for mapping species richness, diversity, biomass or the abundance of different groups of organisms, in both terrestrial and aquatic ecosystems (e.g. Joy and Death, 2004; Bucas *et al.*, 2013; Lopatin *et al.*, 2016). The statistical approaches used for the purpose include more traditional regression-based techniques, such generalized linear models, generalized additive models, and multivariate adaptive regression splines, as well as novel machine learning (ML) algorithms, such as support vector machines, boosted regression trees and random forests. Previous experience has shown that ML techniques can be much more flexible than conventional parametric models due to their ability to handle non-linear relationships and complex interactions, which often occur in ecological data (Guisan and Zimmermann, 2000). Notwithstanding, a review of the literature showed a relatively low number of ML applications in ecology compared with other scientific fields (Olden *et al.*, 2008). Several studies have highlighted the urgent need for comparisons of the performance of different ML predictive techniques in ecology (Aertsen *et al.*, 2011; Olaya-Marín *et al.*, 2013).

The ecology of fish seems to be a promising but little exploited field for the application of ML. This group of organisms is considered an effective indicator of aquatic ecosystem quality due to its sensitivity to anthropogenic disturbances (HELCOM, 2012; Smoliński and Całkiewicz, 2015). Fish species richness and diversity are often used as a primary measures of ecological shifts and as a basis for planning protected areas (Knudby *et al.*, 2010b; Olaya-Marín *et al.*, 2013). Knowledge of the relationships between fish assemblages and environmental factors is important for effective conservation, and the application of novel statistical techniques can improve our understanding of these ecological processes (Olden *et al.*, 2008). A number of predictive analyses on freshwater fish communities, employing ML approaches, can be found in the literature (Olaya-Marín *et al.*, 2013). Most available predictive applications of ML in marine fish ecology refer mainly to coral reef-associated assemblages in shallow waters (Pittman *et al.*, 2007; Moore *et al.*, 2009; Knudby *et al.*, 2010a; Pittman and Brown, 2011). Such works on demersal fish in other ecosystem types are very scarce (Leathwick *et al.*, 2006a; Monk *et al.*, 2010; Compton *et al.*, 2012; Bergström *et al.*, 2013; Bucas *et al.*, 2013).

The aim of this study was to evaluate the performance of conventional regression-based techniques and machine learning used for the predictive modelling of demersal fish diversity. It was assumed that habitat-driven attributes can determine fish assemblage diversity (Knudby *et al.*, 2010a, b; Leathwick *et al.*, 2006b; Pittman *et al.*, 2007). Thus, based on openly available data, we investigated the relationship between demersal fish (species richness and Shannon–Weaver Index) and the environment of the Baltic Sea. The best-performing algorithm was implemented for the spatial prediction of fish diversity from the Baltic Proper to the Kattegat, accounting for the uncertainty of the estimation. We showed how state-of-the-art predictive techniques and simple Geographic Information System (GIS) tools may be applied to obtain reliable spatial information about demersal fish community.

Material and methods

Study area

The Baltic Sea, located in Northern Europe, is a semi-enclosed sea and one of the largest brackish-water basins in the world (415 200 km^2). The study area was located in the south of the Baltic Sea, covering a few distinct sub-basins south of latitude 59°N: Kattegat, Great Belt, The Sound, Kiel Bay, Bay of Mecklenburg, Arkona Basin, Bornholm Basin, Western Gotland Basin, Gdansk Basin, Eastern Gotland Basin, northern Baltic Proper (Figure 1) (HELCOM, 2015). The shallow Danish straits are the only connection with the Atlantic Ocean, where inflows of saline oceanic waters occur. Consequently, a high salinity gradient can be observed within the study area (from ~5 in the northern Baltic Proper up to 27 in the western Danish straits). The Baltic Sea is considered an area of low biodiversity compared with the open oceans and most freshwater environments (HELCOM, 2009).

Fish data

We used data collected during the Baltic International Trawl Survey (BITS) programme in the 1st and 4th quarter of the years 2001–2014. To minimize the effect of variation in gear, we included in the analysis surveys conducted with two standard bottom trawls: TV3 930 meshes (TVL) and TV3 520 meshes (TVS) (ICES, 2011). Fish data were collected by different scientific institutions located around the Baltic and combined by the International Council for the Exploration of the Sea (ICES) in the Database of Trawl Surveys (DATRAS), which is freely available through the ICES website. In the analysis, we included only hauls where all species in the catch were recorded. We merged two datasets: the exchange data, containing detailed haul information, and the data on catch per unit effort (CPUE) per species and length group for each haul, comprising an indirect measure of the fish abundance of every species in different length classes.

The scope of investigation was the alpha-diversity (Foley *et al.*, 2010) of the demersal fish community, so we determined the allocation of all fish species to different ecological groups according to the information available on the FishBase.org website (Froese and Pauly, 2015) and excluded all pelagic fish. Herring (*Clupea harengus*), defined on FishBase.org as benthopelagic, was also excluded from the analysis because its Baltic population is

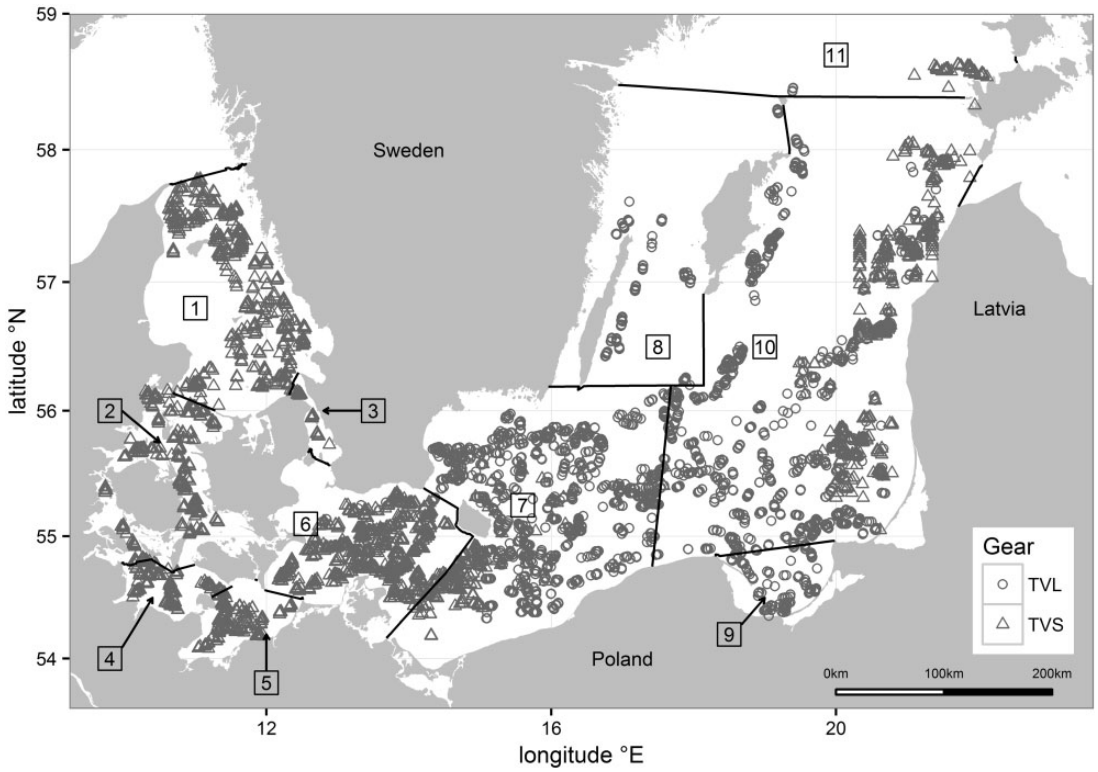

Figure 1. Fish sampling site locations. The shape of the points indicates the gear type used for trawling, lines show borders between HELCOM sub-basins: 1—Kattegat, 2—Great Belt, 3—The Sound, 4—Kiel Bay, 5—Bay of Mecklenburg, 6—Arkona Basin, 7—Bornholm Basin, 8—Western Gotland Basin, 9—Gdansk Basin, 10—Eastern Gotland Basin, and 11—northern Baltic Proper.

Table 1. List of predictors used for analysis of demersal fish diversity.

Predictors	Data type	Range	Data source
Haul data			
Quarter	Factor, 2 levels		(ICES, 2011)
Trawling gear type	Factor, 2 levels		(ICES, 2011)
Modelled environmental data (GIS layers)			
Bottom salinity	Factor, 5 levels		(Al-Hamdani and Reker, 2007; HELCOM, 2015)
Depth	Continuous	9.4–218.1 m	(Seifert *et al.*, 2001; HELCOM, 2015)
Seabed slope	Continuous	0–6.8%	(Al-Hamdani and Reker, 2007; HELCOM, 2015)
Growth season bottom temperature	Continuous	3.9–16.8 °C	(Al-Hamdani and Reker, 2007; HELCOM, 2015)
Seabed sediments	Factor, 5 levels		(Al-Hamdani and Reker, 2007; HELCOM, 2015)
Annual mean bottom current velocity	Continuous	0–0.13 m s^{-1}	(Al-Hamdani and Reker, 2007; HELCOM, 2015)

considered pelagic (Cardinale, 2000). In total, 88 species occurred in the dataset. The species diversity of the fish community was quantified using two indices: species richness and Shannon–Weaver (which combines species richness and evenness). We defined the species richness for each haul simply as the number of species in the catch. We calculated Shannon–Weaver Index of diversity based on the log $(x+1)$ transformation of the relative abundance of each species in the catch. Preliminary analysis showed that haul duration was a significant factor for observed species richness (increasing trend), so we included in the analysis only trawls with a standard duration of 30 min (5715 hauls in total) (Figure 1). Among the haul descriptors, we considered only the season of the survey (1st or 4th quarter) and the trawling gear type (TVL and TVS) as potential predictors of demersal fish diversity (Table 1).

Environmental data

We hypothesized that different features of marine habitats can influence the demersal fish community. We used maps of selected modelled physical features derived by the Baltic Interreg project BALANCE (Al-Hamdani and Reker, 2007) and a depth relief map (Seifert *et al.*, 2001) available from the HELCOM Baltic Sea data and map service (HELCOM, 2015) to provide environmental information for modelling demersal fish diversity and species richness. The selection of variables was based on the literature, expert knowledge and data availability. The list of predictors considered in the analysis is presented in Table 1. The GIS raster layers used in the study were resampled with bilinear interpolation or the nearest neighbour method to the cell size of 100 m and the same resolution was used for prediction. The range of GIS layers covered the whole Baltic Sea, but we intentionally reduced the

geographical scope of the analysis and set the northern edge to latitude 59°N, with regard to the distribution of fish sampling sites. Because no trawls were conducted in the regions of water bottom salinity specified originally in the model as Oligohaline I (salinity <5), we combined all areas with salinity <7.5 (originally classes Oligohaline I and Oligohaline II) into the first class (I) in our dataset. The maps used in the study are shown in the Supplementary Data for this article.

Data analysis

We performed all data exploration, development of different models, GIS analysis and predictive mapping using the *R* scientific computing language (R Development Core Team, 2011). We employed the Classification and Regression Training package *caret* (Kuhn, 2008) with the range of add-on packages for model building, tuning and accuracy assessment. Six alternative predictive techniques, listed in the following sections, were evaluated in this study. The *caret* package allowed us to train different algorithms in a consistent environment and to conduct direct comparisons of model performance. Moreover, internal tuning made it possible to optimize the model parameters, especially of the machine learning. We used 10-fold cross-validation resampling method to assess each model's accuracy (Hastie *et al.*, 2009). We additionally implemented a bootstrap approach by repetition of the whole cross-validation process 100 times with independent resampling of subsets, achieving a total of 1000 permutations for a single model. The results from the folds in each repetition were combined to select the model with the best parameters and to compare different algorithms. As the measure of model performance, we used the root-mean-squared error (RMSE) of the prediction estimated for each repetition. Because we conducted parallel tests for all modelling techniques, using the same test and training sets, detecting pairwise differences in the performances of alternative predictive techniques was a paired-sample problem (Knudby *et al.*, 2010a, b). Therefore, we applied pairwise comparisons using paired *t*-tests with the *p* value adjusted by the method of Benjamini and Hochberg (1995) to control the false discovery rate (FDR), the expected proportion of false discoveries amongst the rejected hypotheses. We evaluated the performance of the finally selected model by calculating RMSE of predictions for the whole study area and for each sub-basin.

Regression-based techniques

Herein, both generalized linear models (GLMs) (McCullagh and Nelder, 1989) and generalized additive models (GAMs) (Hastie and Tibshirani, 1990) were used for the predictive modelling of demersal fish community indices, using an appropriate family of distributions. Species richness data were treated using the Poisson distribution, while the Shannon–Weaver Index, due to its normal distribution, was analysed using a model based on the Gaussian law. We fitted separate models for both indices and all predictors, using, respectively, the basic *glm* function of *R* (R Development Core Team, 2011) and the *gam* function of the *mgcv* package (Wood, 2001). All variables were included in the analysis, since the generalized variance-inflation factors (GVIF) showed acceptable level (GVIF < 5) of predictors' collinearity. We used multiple smoothing parameter estimation by Generalized Cross Validation (GCV) with default gamma and 9 degrees of freedom allowed as maximum for the continuous predictors in GAMs. Additionally, Akaike's Information Criterion (AIC) was used by the *stepAIC* procedure of the *MASS* package (Venables and Ripley, 2002) for the stepwise feature selection of the GLMs.

We also applied multivariate adaptive regression splines (MARS) (Friedman, 1991) with implemented bagging procedure. We used the *bagEarth* function of *caret* based on the *earth* package (Milborrow, 2015). After preliminary tuning of the model, we set maximum number of terms in the pruned model to 14 and the maximum degree of interaction to 2.

Machine learning

In this study, support vector machines (SVM) (Cortes and Vapnik, 1995) based on the radial basis function (RBF) kernel was used. In the *caret* environment, the parameter σ was estimated using the *sigest* function of the *kernlab* package (Karatzoglou *et al.*, 2015), and the parameter C was tuned when running the algorithm.

To develop the boosted regression trees (BRT) (Elith *et al.*, 2008) we used the *gbm* package (Ridgeway, 2007). The values of the two BRT parameters were chosen from defined ranges in *caret* using a cross-validation method: the interaction depth (here 1, 3 or 9) and the number of trees (here 50–1500 in step of 50). We set the two remaining parameters manually as follows: minimum number of observations in the trees terminal nodes = 20 and shrinkage = 0.1.

The random forest (RF) (Breiman, 2001) model was developed using the *randomForest* package (Liaw and Wiener, 2002). We used cross-validation to check the performance of RF with one to eight variables randomly sampled as candidates at each split during the model building. The importance of variables was evaluated by the total decrease in node impurities from splitting on the variable, measured by the residual sum of squares and averaged over all trees.

Predictive mapping

Finally, we re-fit the best model with the whole dataset and optimal parameter values defined in the cross-validation process. We used this model further for predictive mapping of the demersal fish diversity indices, based on the GIS-layers of environmental data and fixed values of two haul factors (4th quarter and TVL gear type). In MSP activities, confidence in the predictions is crucial, and an appropriate presentation of uncertainty is important for decision-makers to understand data reliability (Caldow *et al.*, 2015). We obtained information about prediction uncertainty for each cell of produced map. In the case of linear models, bootstrap techniques can be used, while ensemble approaches such as boosted regression trees and random forests allow direct measurement of the standard deviation of predictions obtained from all the trees used for the final prediction (mean value of these trees). The coefficient of variation was calculated to provide a visualization of spatial regions with higher uncertainty levels. For that purpose, we used the *ModelMap* package, which makes it possible to read large GIS data in sections and maintain a reasonable usage of computer memory (Freeman *et al.*, 2010). This approach gave quantitative information on prediction validity, which was reduced into four qualitative categories (ranked according to the quartiles of the coefficient distribution) to enhance interpretability and map clarity.

Results

Fish assemblage indices

The species richness of the demersal fish communities in the analysed dataset ranged from 1 to 26 per haul (mean 5.8, standard deviation 4.2), and the Shannon–Weaver Index ranged from 0 to 3.03 (mean 1.39, standard deviation 0.66). We noticed a significant relationship between these two indices of the fish assemblages. For this reason, the comparison of all tested predictive methods gave the same model rankings for the Shannon–Weaver Index and species richness, with similar relative performance of particular techniques. Therefore, we decided to show only the results of the Shannon–Weaver Index modelling (see Species Richness Results in Supplementary Data).

Model performance evaluation

The predictive performance of the models is shown in Figure 2. The results of the repeated cross-validation indicate differences in the performance of the tested modelling approaches. ML methods, especially tree-based ensemble models, outperform regression-based techniques in terms of prediction accuracy. The differences in prediction errors obtained for each model by cross-validation were significant (the *t*-test adjusted *p* value for all models pairs was <0.001). The lowest RMSE was found for random forest, while the highest errors were observed for GLM. All tested models showed similar stability of the measured errors (the RMSE standard deviation ranged from 0.013 to 0.014).

Random forest

Random forest, as the algorithm with the best performance among the tested methods, was selected to predict the diversity of demersal fish. The results of the tuning process for the random forest parameter *mtry* showed that the optimal number of predictors randomly selected during the growing of each tree is 5.

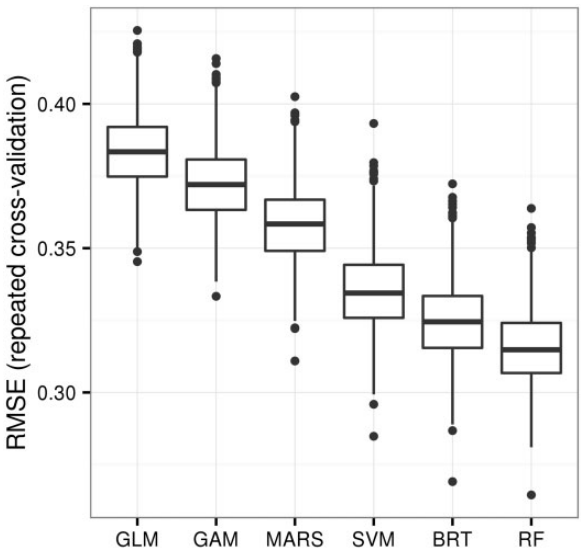

Figure 2. Root-mean-square error (RMSE) as predictive performance measure of the six selected modelling techniques for the Shannon–Weaver Index of the demersal fish community. Cross-validation (10-folds) with 100 repetitions was used for testing. Lines, boxes, and whiskers are medians, interquartile range (IQR), and 1.5× IQR of the estimated RMSE, respectively.

The addition of more variables does not increase the accuracy. The constructed random forest included 500 trees and explained 77.05% of the variance in the data. The model's predictive precision seemed to be better for the higher values of Shannon–Weaver Index, but slight underestimation was observed in the upper ranges. Conversely, the predicted values obtained from random forest were overestimated for observations when only one species was recorded and the Shannon–Weaver Index equalled zero. RMSE of predictions calculated for each sub-basin of the study area ranged from 0.08 in the Kattegat ($n=507$) to 0.31 in the northern Baltic Proper ($n=58$) (Table 2).

The salinity class was the most important predictor of demersal fish diversity (relative importance 42%), followed by depth (23%) and mean bottom temperature during the growth season (14%). The gear type and season of survey showed low levels of relative importance, 7% and 3%, respectively (Figure 3a). Partial dependence plots indicate that the Shannon–Weaver Index of the demersal fish community increases with salinity (Figure 3b). Furthermore, non-linear relationships can be observed between predictors expressed in a continuous scale (depth, bottom temperature, bottom current velocity, seabed slope) and response variables (Figure 3c–f). The local maximum of the Shannon–Weaver Index occurred in localities with ∼40 m depth and the highest observed bottom water temperatures. The partial dependence plots also show that fish diversity increases with increasing annual mean current velocity, up to ∼0.06 m s^{-1}, and remains stable for the higher values.

Predictive mapping

The modelled spatial distribution of the Shannon–Weaver Index highlighted a strong gradient of demersal fish diversity within the studied area, connected with high natural salinity differences in the Baltic Sea (Figure 4a). In geographic terms, the highest predicted Shannon–Weaver Index is observed in the Danish Straits and decreases with increasing distance from the Straits. The pattern of fish diversity distribution is also strongly influenced by the bathymetry. The lowest predicted Shannon–Weaver Index occurred in the deepest waters of the Baltic, and higher values are predicted along the coastline.

The prediction map is supported by the information on the uncertainty level obtained from the results of all 500 trees combined in the random forest model. The coefficient of variation was calculated for each cell in the map and ranked with

Table 2. Number of observations (*n*) and root mean square error (RMSE) of Shannon–Weaver random forest predictions calculated for sub-basins in the study area.

Sub-basin name	*n*	RMSE
Arkona Basin	975	0.10
Bay of Mecklenburg	202	0.16
Bornholm Basin	1541	0.16
Eastern Gotland Basin	1518	0.17
Gdansk Basin	140	0.17
Great Belt	358	0.13
Kattegat	507	0.08
Kiel Bay	169	0.16
Northern Baltic Proper	58	0.31
The Sound	72	0.11
Western Gotland Basin	175	0.18

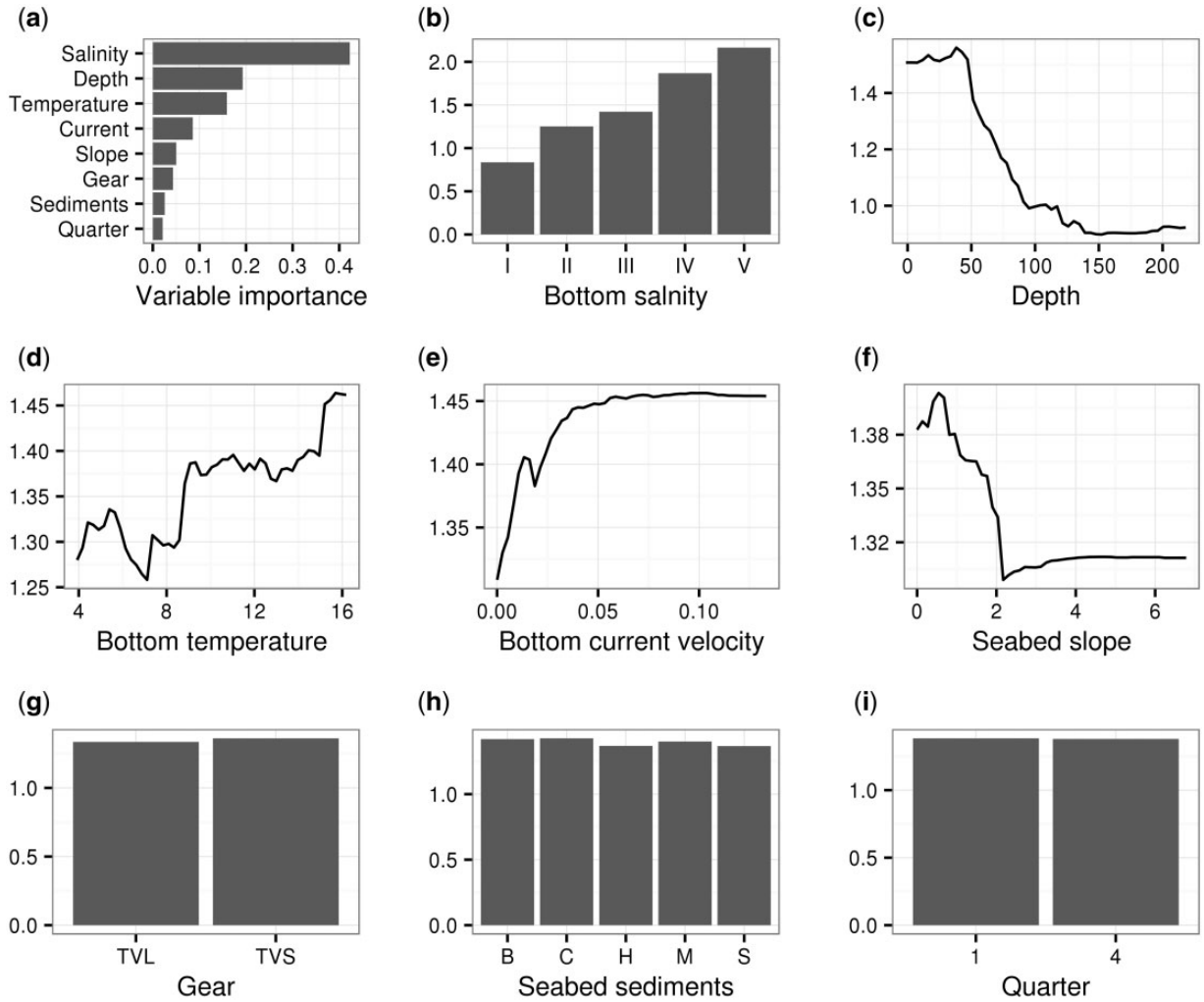

Figure 3. Relative importance of predictors expressed as % contribution in the model (a). Partial dependence plots for random forest regression of Shannon–Weaver Index and eight predictor variables (b–i). Partial dependence plots show the dependence of the response variable after marginalizing the effects of the other predictors. Variables are ordered by decreasing importance.

thresholds of classes calculated according to the quartiles of the coefficient distribution (Table 3). The final uncertainty map is presented in Figure 4b and shows that the prediction precision in the studied area varies due to unequal coverage of the collected samples and model abilities. The highest values of Shannon–Weaver Index were predicted with relatively low variation, which agrees with the results obtained during model evaluation. An unacceptable level of model precision (coefficient of variation >59%) occurred in the regions with low predicted diversity, especially the deeper areas, while in shallow waters much lower variance was observed.

Discussion

Predictive modelling has been widely used for mapping species richness in aquatic ecosystems by applying traditional regression-based techniques (GLM, GAM, MARS), but the application of machine learning in marine fish ecology is, to our knowledge, still surprisingly scarce. In particular, in the Baltic Sea area, comprehensive works on species modelling were conducted using maximal entropy (MAXENT), GAM and RF models to evaluate eutrophication management scenarios provided by the Baltic Sea Action Plan (Bergström *et al.*, 2013). The quoted studies, however, aimed to aggregate the forecasts of the applied methods rather than to compare them. The results showed that the combined models can be successfully used to predict perch (*Perca fluviatilis*) and pikeperch (*Sander lucioperca*) recruitment areas with respect to the changes in water transparency projected by eutrophication scenarios. The general findings of Bergström *et al.* (2013), indicating valuable properties of combined predictive techniques, outline further possible directions for the works presented herein. The ensemble approach can be used to minimize model-specific errors in predictions of species distribution.

Furthermore because the extent of predictive maps produced in our study was arbitrarily reduced with respect to sample coverage, more effort should be applied to testing the transferability of the developed models and their abilities to enlarge the mapped area. For example, the next step could be to test the models in areas further north in the Baltic Sea, outside the sampling coverage. The model applicability might differ considerably if the extension of the predicted area encompassed Baltic Sea bays, e.g. the Bothnian Bay, which constitutes a large part of the sea with a distinct environmental character (HELCOM, 2009). As shown by

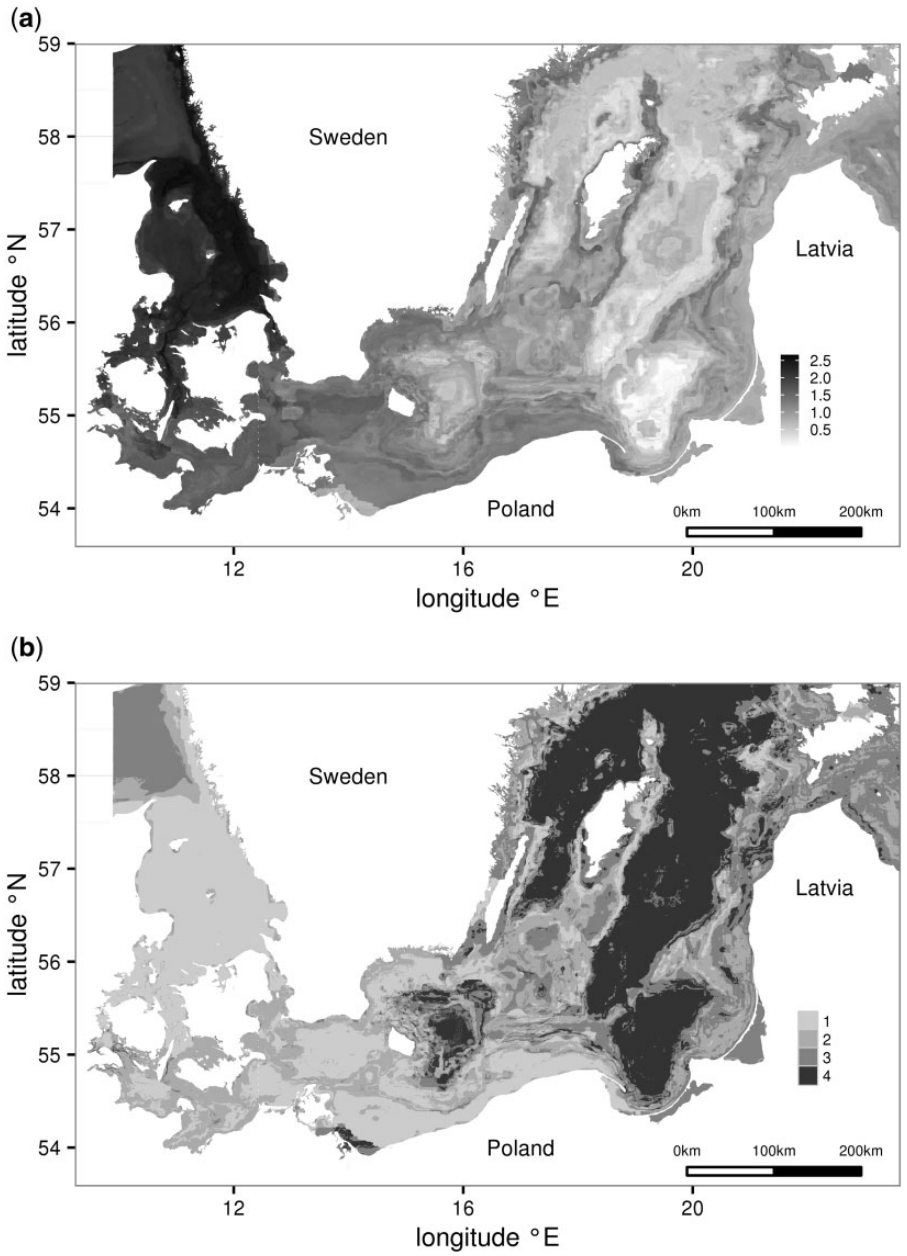

Figure 4. Map of predicted Shannon–Weaver Index of demersal fish community for the 4th quarter and TVL gear type using random forest model (a). Map of prediction uncertainty categorized with four levels, as specified in Table 3 (b).

Table 3 Thresholds for coefficient of variation used for assignment uncertainty levels of Shannon–Weaver Index prediction.

Coefficient of variation [%]	Rank of uncertainty
<28	1
28–41	2
42–59	3
>59	4

Sundblad *et al.* (2009), both differences in the observed range of the predictor variables in studied localities and the direct–indirect nature of the variables' effects should be considered in such investigations.

We identified the salinity class as the most powerful predictor of demersal fish diversity (relative importance 42%), followed by depth (19%) and mean bottom temperature during the growth season (16%). The remaining predictors were of low importance (<10%). Similar findings were presented by Leathwick *et al.* (2006a). They concluded that the high level of predictability was caused by the strong relationship between demersal fish species richness and the environment, with particular emphasis on depth and temperature. Depth of the oceanic region described in the paper by Leathwick *et al.* (2006a) varied from 5 to 1700 m. Remarkably diverse depth drives demersal fish species richness variability. Analogously, depth, coarse-scale topographic complexity measured by depth range or rugosity were the most important factors determining the diversity of coral reef-related fish

communities, although the depths did not exceed 8 m (Knudby *et al.*, 2010a, b). The results of the two quoted studies highlight depth as the strongest predictor, while in our study it was salinity, which results from the strong salinity gradient observed in the Baltic Sea, in contrast to the other discussed areas. It should be noted, that the depth effect in our study area may be masked by salinity, which varies considerably (approximately between 5 and 27) in comparison with stable salinity observed in the oceans surrounding New Zealand (differences <1) (Leathwick *et al.*, 2006a). Our results were in line with studies conducted by Snickars *et al.* (2014), who demonstrated high importance of salinity as predictor of fish distribution in the Baltic Sea.

Partial dependence analysis indicated a general increase in the Shannon–Weaver Index of the demersal fish community with increasing salinity, while for depth and bottom temperature, local maxima of the Shannon–Weaver Index were observed at the depth of ∼40 m and at the highest temperatures. Higher temperature and seasonal heat of water masses may lead to changes in stratification and the characteristic of food webs, e.g. by prompting biological production and increasing fish diversity (Mackenzie *et al.*, 2007). It is important especially in the case of the shallow waters of the Baltic and the Kattegat, warming up rapidly during the summer. The lowest diversity found in the deeps is caused by oxygen deficiency and anoxia, which affect fish distribution directly and indirectly by limiting prey availability (Mackenzie *et al.*, 2007). We may conclude that the environmental relationships of the Baltic Sea demersal fish diversity envisaged by the random forest method were mostly consistent with the available literature, showing high importance of hydrographical features as the predictors of fish distribution (Snickars *et al.*, 2014). However, Snickars *et al.* (2014) reviewed studies on species–environment relationships focusing on benthic organisms in the coastal areas of the Baltic Sea, finding hydrography (salinity and Secchi's depth) and biotic features to be the most widely used predictors of benthic fish. Their conclusions implied our suggestion to consider other potential environmental parameters in further model development: e.g. no information on water transparency or biotic traits was included in the predictive modelling presented herein. Water transparency in shallow waters is related to productivity and thus may affect both juveniles and adult fish by food supply (e.g. higher invertebrates density) (Snickars *et al.*, 2015). Besides the above-mentioned indirect effect, also direct impact is observed, as water transparency may affect the prey capture rate, predator avoidance or habitat choice of fish (Sundblad *et al.*, 2009).

From the methodological perspective, Lopatin *et al.* (2016) concluded that GLMs fitted for vascular plant species richness delivered better results in terms of precision and bias than RF, as GLMs can handle count data with better performance. However, in our case study, ML methods, especially tree-based ensemble models, surpassed regression-based techniques in terms of prediction accuracy. Similarly, results of studies by Bucas *et al.* (2013) pointed out RF as the most accurate method, when comparing with GAM and MAXENT. In general, the differences in prediction errors obtained for each model by cross-validation were statistically significant. The RF method revealed the lowest RMSE value among the models considered. In addition, a high level of agreement ($R^2 = 0.95$, $p < 0.001$) was demonstrated between the observed and predicted values of the Shannon–Weaver index for the RF model. The comparison of RMSE for RF models with different numbers of randomly selected variables (parameter *mtry*) with RMSE of other algorithms showed that the inappropriate tuning of model parameters can change the final decision on modelling method selection. For example, the use of $mtry = 3$ in RF building negatively influences the model performance and results in slightly worse accuracy than for the BRT method. The predictive precision of properly tuned RF seems to be better for higher values of the Shannon–Weaver Index. This feature of the fitted model is considered to be desirable, as the areas of higher biodiversity are of the greatest interest in a spatial management context (Roberts *et al.*, 2002). Differences in RF precision were also evident on sub-basins level. It was revealed by the lowest prediction error obtained for the Kattegat, where predicted fish diversity was the highest. In contrary, RMSE obtained for the northern Baltic Proper exceeded RMSE achieved for all the other sub-basins. It may result both from low number of observations ($n = 58$) and low diversity in the northern Baltic Proper. The robustness of the model and the statistical properties of the random forest indicated its selection for the prediction of demersal fish diversity in our investigation.

Any results of modelling should be interpreted with caution, taking into account the uneven distribution of sample coverage, which may have consequences in terms of prediction uncertainty (Young and Carr, 2015). The BITS are designed to obtain representative abundance indices of the most important bottom commercial species (cod and flounder). However, the data collected during the surveys give good opportunity to use them also for biodiversity modelling, as all species in the catch are recorded. The BITS haul allocation is random, but the coverage is constrained to trawlable areas (ICES, 2003). Consequently, some areas, e.g. with complex hard-bottom are permanently unsampled. Fish communities occurring on the areas of heterogonous seabed, which are underrepresented in the DATRAS, are commonly more diverse than soft-bottom assemblages. This implies that developed models may not be capable of handling variability of fish diversity in the small scale, governed mainly by local biotic factors (Florin *et al.*, 2009). Also deeps and shallow waters are not fully monitored (the distance between sampled locations may in some areas reach dozens of km). However, in the case of Baltic Sea deeps, it is assumed, that fish fauna is absent in oxygen depleted bottom zones (ICES, 2011). On the contrary, fish are usually more diverse in shallow waters, than in the open sea demersal zones (HELCOM, 2012), but the depths up to 10 m are not sampled during BITS, due to dimensions of the standard trawls and research vessels. In addition, the data used in the study are constrained to quarter 1 and 4. Consequently some fish migrations (e.g. spawning or feeding) are not incorporated in the presented model. The shortcomings of the data mentioned earlier, imply that time and spatial fish diversity patterns incorporated in the models are incomplete in the context of the whole year and in some Baltic Sea areas, but still may act as a useful surrogate for predicting fish diversity (Pittman *et al.*, 2007).

The environmental data used in the study were represented in the high spatial resolution, providing spatially detailed information about the distribution of selected physical features in the Baltic Sea (Al-Hamdani and Reker, 2007). Our approach was relatively simplistic, because we used static environmental variables that did not represent changes over several years, in which fish data were collected. For example, evident shifts in the distribution of anoxic and hypoxic waters have been observed during recent decades in the Baltic Sea, which might affect spatial patterns of fish diversity (Mackenzie *et al.*, 2007; Hansson and

Andersson, 2013). Moreover, some of the modelled environmental data do not take into account seasonal variability. The fish data from BITS survey were collecting during cold 1st and 4th quarter, while the information about modelled bottom temperature of water referred to the warm growth season. Furthermore, salinity as one of the most important factors affecting fish diversity was expressed in our study as discrete variable with five classes. The application of more detailed, continuous variable may improve accuracy of model. Besides the limitations, the use of these basic environmental data in the modelling of fish–habitat relationships allows to conduct prediction over broad spatial scale and obtain rapid and cost-effective information about the fauna distribution (Pittman *et al.*, 2007).

Our study showed the feasibility of applying novel statistical approaches to the spatial prediction of fish assemblage diversity. Based on the presented results, we share the opinion expressed in Bolker *et al.* (2013) that ecologists should be able to implement models both within and outside traditional statistical frameworks that seem suitable for their specific scientific investigations. This ability is particularly important now, as the dynamic development of natural resource management and conservation moves towards more spatial and ecosystem-based approaches (Crowder and Norse, 2008), which often require more flexible methods of biological data analysis (Elith *et al.*, 2008). The application of machine learning, such as random forests, may be valuable for obtaining knowledge on biotic and abiotic factors affecting species distribution and precise, quantitative information on spatial variation in species diversity, which are essential for management actions (Young and Carr, 2015). Our comparative work highlighted the potential of machine learning method to reduce prediction error in modelling of demersal fish diversity. More accurate models of marine fauna–environment relationships should improve predictive maps of species distribution used in MSP and in consequence provide more reliable qualitative basis for management of marine areas or strategies for biodiversity conservation.

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Supplementary data

Supplementary material is available at the ICESJMS online version of the article.

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Appendix 15.

Metadata sheet

No	Descriptor	Information
1	Title of dataset	Baltic Sea mesozooplankton dataset
2	General description	Harmonized dataset from institutional monitoring programs of several countries around Baltic Sea
3	Keywords	Baltic Sea, mesozooplankton, monitoring
4	Data type (observational/model)	Observational
5	Parameters	Taxonomic composition and abundance of mesozooplankton (incl rotifers, cladocerans, and copepods)
6	Area covered	Baltic Sea
7	Spatial resolution	A few kilometres
8	Time span	1957-present
9	Temporal resolution	From daily to monthly
10	Entry date	13. January 2017
11	Institution	Estonian Marine Institute, University of Tartu
12	Originator	Riina Klais
13	Originator e-mail	Riina.klais@ut.ee
14	Publication where data used	Klais, R., Otto, S., Teder, M., Simm, M. and Ojaveer, H. Winter-spring climate effects on small-sized copepods in the coastal Baltic Sea. ICES JMS (conditionally accepted)
15	Location of dataset	Contact originator Riina Klais
16	Availability	Data available according to the network data policy (http://kodu.ut.ee/~riina82/policy.html)

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Appendix 16.

Metadata sheet

No	Descriptor	Information
1	Title of dataset	Herring and three-spined stickleback feeding in the Gulf of Riga
2	General description	Fish were collected during pelagic fish hydroacoustic surves (264 trawls) by a pelagic commercial trawl in 30 min hauls at 3 knots speed. Stomachs of 20 randomly chosen fish per haul were collected for adult and juvenile herring, and three-spined stickleback. Zooplankton samples were obtained from each trawling station by vertical tows of Juday net.
3	Keywords	Diet composition, feeding activity, diversity of stomach content
4	Data type (observational/model)	Observational
5	Parameters	Zooplankton abundance and biomass by species and stations. Qualitative and quantitative feeding of fish by hauls: biomass proportion and frequency of occurrence of different prey taxa in stomachs, number of taxa in stomachs, percentage of empty stomachs, stomach fullness index.
6	Area covered	Gulf of Riga
7	Spatial resolution	In the range of kilometres
8	Time span	1999-2015 (late July – early August)
9	Temporal resolution	One survey lasted for about one week
10	Entry date	13. January 2017
11	Institution	Estonian Marine Institute, University of Tartu
12	Originator	Riina Klais
13	Originator e-mail	Riina.klais@ut.ee
14	Publication where data used	Ojaveer, H., Lankov, A., Teder, M., Simm, M. and Klais, R. 2016. Feeding patterns of dominating small pelagic fish in the Gulf of Riga, Baltic Sea. Hydrobiologia (DOI 10.1007/s10750-016-3071-5)
15	Location of dataset	Contact originator Riina Klais

16	Availability	Data availability according to the data policy of the University of Tartu

Acknowledgements

The research leading to these results is part of the BIO-C3 project and has received funding from BONUS, the joint Baltic Sea research and development programme (Art 185), funded jointly from the European Union's Seventh Programme for research, technological development and demonstration and from national funding institutions.